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THE MALE SEX HORMONES

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The general effects of castration, in addition to the loss of spermatogenesis, have been known since our earliest historical records. It is not the purpose of this paper to review the earlier studies which emphasize the effects of castration of the male, of transplantation of gonads, and of cryptorchidism on the various physiological, psychical, morphological, and histological changes in the body as a whole and in secondary sex characters and organs. Excellent reviews on the older general and more purely morphological aspects have been written by Lipschütz (1924), Pezard (1928), Sand (1926, 1932, 1933), Moore (1932) and Steinach (1936). From these and numerous other studies some have concluded that the hypothetical internal secretion of the testicle must have a profound effect on skeletal growth, muscular tone, metabolic rate, hemoglobin and oxidase content of blood and tissues as well as on the control of the secondary sex characters and accessory reproductive organs of the male. This review shows that only very few of these physiological reactions are sufficiently rapid and definite to be used as assay methods in the purification and isolation of the male sex hormones.

The action of testis-tissue extracts and concentrates on the rate of metabolism. Brown-Séquard and others have associated the fatigue and "lowered vitality" of advanced age with loss of the internal-secreting function of the testis; hence the early claims of rejuvenation by a hypothetical testicular hormone. The experiment carried out by Brown-Séquard (1889) upon himself is not only the earliest of its type in the field of the testicular hormone but in endocrinology in general. At the age of 72 he prepared very dilute and crude aqueous extracts from dog or guinea pig testes and injected them in 1 cc. amounts subcutaneously into himself, irregularly over a period of two weeks. He claimed a remarkable return of physical and mental endurance and of normal

intestinal function which lasted for four weeks. He admitted the possibility of suggestion having been the main factor in his case. Equally unconvincing were the ergographic investigations conducted by Fritz Pregl (1896) and Zoth (1898) upon themselves with glycerol and "Brown-Séquard extracts" of testis tissue. These claims have never been confirmed by others even with more concentrated and more carefully prepared extracts. Nevertheless these observations received remarkable publicity and even today the general claims of action on metabolic rate and "well-being" are still assumed by some. Weil (1920) concluded that the injection of an acid-aqueous extract equivalent to 30 grams fresh testis tissue per kilogram body-weight produced increased CO_2-production in guinea pigs, especially when he used immature or castrated males. He found ovarian extracts to produce the opposite effect. Aude's (1927) studies on capons and cockerels indicated a fall in basal matabolic rate following castration and a rise following the intraperitoneal injection of crude, unconcentrated testis-tissue extracts. He could not observe an effect on the rate of regression or a stimulation of growth of the capon comb in parallel with these effects on metabolism. Hence he concludes that the oxidation stimulant and the hormone acting on the secondary sex characters must be two different substances. Kuznetzov (1928) likewise reports no effect on the capon comb but a tonic effect upon the cardiac muscle, blood vessels, and on the metabolic processes with Kravkov's perfusate prepared from bull testes. Ptaszek (1928) concludes from experiments on two dogs that castration is followed by a lowered metabolic rate, then by an adjustment to a higher level due to overactivity of other endocrines and lastly by a continuous lower rate. In the last condition he reports a rise in metabolism after six injections of "testis-tissue extract." Korenchevsky and associates (1921, 1925, 1928) report that crude emulsions from testis or prostate tissue increased nitrogen excretion in two castrated dogs, but that the gaseous exchange data were contradictory. On the basis of scanty and variable results, they nevertheless conclude that the prostate extract affects protein metabolism, but that the testis acts as a synergist. These studies were repeated on rabbits, normal, castrated, and thyroidectomized, with several types of extracts. With saline "emulsions" of rabbit testes or kidney, they claim, on the basis of very irregular results, a lowered nitrogen metabolism but with prostate "emulsion" alone or combined with the testis preparation, an increased nitrogen excretion. These effects from kidney or testis extracts alone were found more marked in the thyroidectomized than in the normal or castrated rabbit,

but the prostate extract alone did not raise the nitrogen excretion in the thyroidectomized animals. In the later studies, Korenchevsky (1928) at one time limits the protein-metabolism-stimulating action to crude lipin fractions from bull testis and prostate and in the next paper in the same issue, Korenchevsky and Young (1928) find a metabolism-stimulating activity in an aqueous filtrate from testis tissue which is free from the synergistic action on prostate extracts. Korenchevsky (1926) also reports that castration combined with thyroidectomy in two rabbits results in a lethal fall in body temperature when the animals are cooled and hence he suggests that the gonads are involved in the regulation of body temperature. His reports (1930) that cryptorchidism in rats leads to a less marked response, but nevertheless a response in the same direction in the atrophy of accessory sex organs, in thyroid atrophy and in obesity development are suggestive of two hormonal factors, one from the seminiferous elements and the other from the Leydig cells, or of a gradual degeneration of the entire interstitial tissue. He (1930) found indications that cryptorchid pigs, however, gain weight faster and deposit more retroperitoneal fat than the castrated controls. He (1932) further observes that during the early stages of cryptorchidism in rats, there exists a higher metabolic rate as measured by gaseous exchanges; from this he concludes that the stimulation probably arises from the rapid resorption of the seminiferous cells. Recent studies by Holt, Keeton, and Vennesland (1936) do not confirm Korenchevsky's observations on obesity development in castrated male rats. In fact, Gertrude von Wagenen (1928) actually observed lowered body weights in castrated male rats. She confirmed Moore's (1922) earlier observations that castration usually leads to animals having shorter than normal bodies.

From *in vitro* studies on tumor tissues, Nakamura and Zuzuki (1930) conclude that crude sex-hormone preparations increase the respiration. Wu (1936), however, finds that the respiratory activity of cerebral sections is not altered by castration.

The studies above are at best only suggestive of a testis-tissue humoral control in basal metabolic rate and growth. The biological effects are not uniform and definite enough to encourage one to undertake these criteria for the necessary systematic quantitative fractionation of the very crude extracts used thus far. Furthermore, the slight and doubtful effects observed from extracts may be due to other extractives which may be present in other tissues and therefore are not specific. More recent studies on more definitely purified concentrates of the lipin frac-

tion which have been standardized in terms of capon units on the basis of the comb-growth response likewise lead to inclusive results on metabolism. Bühler (1933), on the basis of very scanty experimental evidence, arives at the most startling conclusion that 50 to 150 capon units of "Proviron" (Schering-Kahlbaum) act specifically on the creatine metabolism in the male by decreasing creatinuria. Kochakian and Murlin (1935) found injections of a male-hormone concentrate from urine to produce a slight increase in fat metabolism, a decrease in protein metabolism (as measured by urea excretion) and no change in the carbohydrate metabolism in a thin castrated dog. In the fat castrated dog, carbohydrate metabolism is decreased. Unpublished data in the author's laboratories, obtained by E. W. Wallace, on castrated rats with highly purified concentrates from human urine and testis tissue respectively in daily doses of 5 to 10 capon units, reveal no effect on the basal metabolic rate.

Detection and assay of androgens by the comb-growth response in the capon. The first and most conclusive experimental evidence that the testicle controls the size of cockerel combs through a humoral mechanism was given by Berthold (1849). In fact, it is the first clear-cut evidence for any internal secretion by transplantation of the organ involved. Berthold even showed that amputated combs and wattles from a capon are regenerated by the transplantation of testes. The first investigator to produce comb growth in normal young hens by the repeated injection of a saline extract from cockerel testes over a period of months was Walker (1908). Pezard (1911) likewise first produced comb growth in black Orpington capons by injecting twice weekly over a period of five months the "extract from one-tenth of a cryptorchid hog testis." In spite of the very small amount of material used, it is worth noting that the long period of injection led to crowing and to sexual behaviour. These studies, although very clear cut, were only of qualitative value and limited in application on account of the time factor involved. It remained for McGee (1927, 1928) to fractionate and concentrate the material so that the substance could be detected in a five-day assay and to show that the activity is found in the lipin fraction from bull-testis tissue. The qualitative and specific distribution of this activity in bull testis and epididymis was shown by Gallagher (1928). The hormone has also been extracted from calf, swine, and stallion testes, but only one positive result has been reported on ram testes (Womack and Koch, 1930). The routine processing of a 500-pound lot of ram testes by Gallagher and Koch led to negative results.

The first quantitative method as devised by Gallagher and Koch (1929, 1930) was based on the minimum dose necessary to produce a detectable comb growth in five days. They succeeded in purifying the active lipin fraction to such a degree that 0.01 mgm. per day for five days produced a detectable comb growth in brown Leghorn capons. Gallagher and Koch (1930) from their quantitative studies concluded that daily injections for five days are more satisfactory than shorter or longer periods for assay purposes, that an increase in length plus the increase in height of the comb of 3 to 7 mm. is a desirable range. They defined the capon unit as the amount which, injected per day for five days, yields an average of 5 mm. increase in length and height of the combs on at least five brown Leghorn capons. They furthermore determined the "characteristic curve" establishing the relation of dosage to comb-growth response. Later experiences have confirmed these findings but have also shown (Womack and Koch, 1930; Womach, Koch, Juhn and Domm, 1931; Koch and Gallagher, 1934) that the comb-growth response value is affected by the intensity of light to which the capons may be exposed and by the initial length of the comb, but that the age of the capon and its repeated use does not influence the response seriously. To correct for the light factor, all assays must, therefore, be conducted in parallel with an assay of a standard preparation. Proper corrections must also be made for the initial comb-length. Unpublished data from the author's laboratory also show that the same "characteristic curve" in comb-growth response holds for the variations in dose of crystalline androsterone, trans-dehydro-androsterone, androstenedione, and androstanediol.

Greenwood, Blyth and Callow (1935), by using essentially the same method of assay, also established the "characteristic curve" for crystalline androsterone. They report that the amount and character of the solvent used influences the response, but that initial comb-length was not a serious factor in their studies.

Freud, de Fremery, and Laqueur (1930, 1931, 1932) measure the comb-growth response by photographing the shadows cast by the comb before and after injection and determining the change in area of the shadows by a planimeter. They define a unit as the minimum daily dose which, introduced in two injections per day for four days, causes on the fifth day, an increase of 15 per cent in the area in over 50 per cent of the capons. To complete an accurate assay by this method may require four to five weeks because the minimum dose must be determined directly and is not calculated from a "characteristic curve." Although

these authors have confirmed the influence of illumination on the comb-growth response, they do not consider it necessary to correct this by assaying a standard in parallel with the unknowns. They also do not correct for the initial comb-size unless the capon weighs less than 1.5 kgm.

Gradstein (1934, 1935) has devised a method based on measuring the comb size by means of the photo-electric cell.

Ogata and Ito (1933) and Ogata, Hirano, and Tonaka (1934) also use a modified planimeter method for measuring the change in area of the photographic shadows of combs. They measure only the trapezium area exclusive of the barbles and thus eliminate serious errors in the planimetric measurements. They attempt to correct for the light factor by keeping the capons in a basement room during the assay period. The effect of the initial comb-size on the growth response is ignored although the data clearly show that this is a factor. The relation of per-cent increase to increase in dose is expressed approximately by a straight line for a range covering 10 to 30 per cent increase in area, but beyond that the curve becomes ellipsoid in shape.

With pure hormone solutions, it is now feasible to apply the hormone solution to the surface of the comb or to inject a sterile solution directly into the comb. By these procedures, the response is much greater per unit weight of hormone (Ruzicka and Tschopp, 1934, and Greenwood and Blyth, 1935).

At the meeting of the League of Nations Committee held in July 1935, the international unit of male hormone was established as 100γ of crystalline androsterone and it was recognized that it is essential to assay all preparations in parallel with a standard androsterone preparation. Henceforth it is very desirable to have all studies expressed in terms of this international unit.

Detection and assay of androgens by the response in the accessory sex organs in mammals. The biological bases of these assays were largely developed as a result of the pioneer castration, transplantation, and cryptorchidism investigations by Steinach (1894) and Moore (1926–1928). The earliest approach to the production of an active extract from testis was reported by Ancel and Bouin (1906) when they injected dilute glycerol extracts of cryptorchid testes for nine months into three castrated guinea pigs and observed the length and weight of the penis, and of seminal vesicles as compared with the length and weights of these organs in normal and untreated castrated animals. Stern and Battelli (1923) likewise made a few observations on the change in the guinea-pig

seminal vesicle as a result of the injection of a testis-tissue perfusate. They themselves say that the experiments were too few "permettre des conclusions définitives."

Spermatozoön motility test. In connection with this test it is interesting to note that Stigler (1918) considered the epididymis to contain a substance which is favorable to the vitality, motility, and "ripening" of the spermatozoa. In 1925 Benoit observed that when an epididymis was ligated in the guinea pig, the spermatozoa retained their motility longer when testes remained than when the animals were castrated. Moore (1922, 1926, 1927, 1928) independently announced the same results in the guinea pig and suggested this phenomenon as a qualitative indicator for the testis hormone. In 1928 Moore and McGee employed

TABLE 1

Comparative capon units of various investigators

LABORATORY	NUMBER OF INJECTIONS PER DAY	DAYS INJECTED	NUMBER OF CAPONS USED	RESPONSE FOR A UNIT	EQUIVALENT IN* INTERNATIONAL UNITS (DAILY DOSES)
Butenandt et al.	1	2	3	20% area increase	1.5–2.0
	2	4	3		
Gallagher and Koch	1	5	5–20	Increase ≏ in L + H of 5 mm.	1.0
Laqueur et al.	2	4	4–12	15% area increase	approx. 1.0
Ogata, Hirano, Tanaka	1	2	3	20% area increase	>2.0
Tschopp	1	6	5	20% area increase	0.7

* The international unit adopted by the League of Nations' Committee in 1935 is 100γ androsterone.

this test on treated and untreated guinea pigs whose testes had been removed, but with the epididymides left intact in the scrotum. The hormone preparations employed represented crude lipin fractions obtained by McGee from bull testes and from bull brain tissue. Only the former prolonged the motility of spermatozoa in the isolated epididymides. Subsequently Moore and Gallagher (1930) determined the minimum dose, expressed as capon units, given daily to retain spermatozoön motility in the properly prepared guinea pig for the 35 days. The results were quite variable. Negative results were reported on 0.009, 0.018, and 0.09 capon unit and positive on 0.04, 0.07, 0.08, 0.12, and 0.17 capon unit per day for 35 days. Expressed in terms of international units, this would call for 0.1 to 0.2 I. U. per day for 35 days.

Obviously this is a very sensitive qualitative test, but it is of no value for quantitative purposes because the time factor is too long and the quantitative differences in individual guinea pigs are extremely variable.

Prostate reactions. This test was first developed on a histological basis by Moore, Gallagher, Price, and Koch (1929, 1930). The complete replacement experiments demonstrated that a perfectly normal rat prostate can be regenerated in 20 days although the animal may have been castrated for 85 to 90 days previously and even before sexual maturity. By the cytological reaction, Moore and Gallagher found this test to detect as little as 0.1 capon unit (as testis-tissue concentrate) if injected daily for 20 days, but for normal cytology it required 2 to 3 capon units per day for 20 days. Hansen (1933) attempted to establish the quantitative value of the cytological test and shortened the period of injection to 10 days. By this modification he could detect as low as 1.5 capon units distributed over the 10 days. The method is, however, not adaptable to quantitative studies because of the remarkable animal variations and the delay due to the histological technique.

On the basis of the linear measurements or the weight of the prostate, the method is much more rapid than the histological procedure, but by no means as reliable as the capon method from a quantitative point of view. The weight of the prostate is a better quantitative measure than the linear measurement. The animal variations are, however, still so great that the quantitative interpretations may be very misleading. Furthermore, the quantitative response is influenced by the amount of estrogenic material and probably by the amount of another synergist which may be present in the unknown. Nevertheless, the value of the information obtained by this method in parallel with the capon method cannot be overestimated. This will be brought out most strikingly in differentiating between various androgens.

Korenchevsky and his associates (1932, 1933, 1935) have published extensively on the rat prostate and seminal-vesicle weights in castrated rats when injected with androgenic substances. They recommend that the rats for comparative studies be from the same litter and state that two for the unknown and two for the control "are sufficient to obtain accurate results." The rats should be castrated not less than 30 days before use and preferably before they are 30 days old. Injections are made twice daily for 7 days. The seminal vesicles and prostate glands are weighed together after 2 to 3 days in Allen's modification of Bouin's fixative. The prostate gland is then weighed separately. For doses of 200, 450, 600, and 900γ androsterone, the per cent increases

in prostate weight over the untreated castrates when calculated per 200γ androsterone were 48, 48, 47, and 51 respectively. The rat unit is recommended as the amount necessary to cause a 40 per cent increase in the prostate weight as compared with the uninjected castrated control rats.

Laqueur, Freud, Dingemanse, etc. (1931, 1934, 1935), have also employed the weight of the prostate gland in castrated rats as a measure of the androgenic action of various preparations. They have not published their method in detail.

The weight method on the prostate response has been investigated in the author's laboratories in collaboration with David Duncan (unpublished) for some time on castrated rats of various ages and on infantile rats. The results are extremely variable thus far.

Seminal-vesicle reaction. As a cytological test this was developed independently by Moore, Gallagher, Hughes, and Koch (1929, 1930) by Loewe and Voss (1929, 1930) and by Martins (1930). Either rat or mouse may be employed. In the castrated rat it is not as sensitive as the cytological prostate reaction. The minimum daily dose for 20 days which can be detected is 0.2 to 0.4 capon unit (as testis-tissue concentrate) and the dose necessary for normal regeneration of the seminal vesicle was found to be 6 to 7 capon units (Moore and Gallagher). Hansen (1933) also studied this reaction more quantitatively and concluded that a fair quantitative response holds over a range of 0.4 to 7.0 capon units per day for 10 days. Martins (1930) applied the test qualitatively on immature male and on castrated adult mice. Loewe and Voss (1929, 1930, 1931) describe their histological grading system in the castrated mouse in detail and claim that the test is very sensitive and very reliable for quantitative assay. The limitations of this method are the same as for the cytological prostate test.

On the linear measurement or weight basis, the seminal vesicle test appears somewhat more satisfactory than the prostate method. The reaction in weight increase is, however, also influenced by the presence of estrogenic material or other synergists. Korenchevsky and his associates (1932, 1933, 1935) have carried out extensive studies on the development of the seminal weight-method. Their technique is the same as on the prostate and the per cent increase in seminal vesicle plus prostate per capon unit of androsterone given daily for 7 days is also 40 per cent over the non-treated castrate. They actually prefer to take the per cent increase in the combined weight of seminal vesicle and prostate than the prostate or seminal vesicle alone because they consider

the increase in prostate weight alone to correspond to the capon test whereas the combined seminal vesicle plus prostate reaction represents the "whole male sexual activity." Needless to say such statements are very confusing. This is particularly true in view of the synergistic action of estrogenic and other substances. Laqueur and his associates (1931, 1934, 1935) have used an unpublished method based on the seminal-vesicle weights in distinguishing between different natural forms of androgenic substances. Ogata and Hirano (1934) call a rat unit the quantity of material which, in three daily injections, causes a 50 per cent increase in weight of the seminal vesicle over that of the untreated castrated rat. Tschopp (1935) castrates rats at 70 to 80 grams body weight. Four weeks later they are injected daily for 20 days. The average weights of the seminal vesicles from 3 to 10 rats are taken for each dosage of the male hormone. By his method the increase in weight of the seminal vesicles does not increase proportionally at the same rate for different artificially prepared male hormones. Tschopp in fact shows that crystalline theelin in daily doses of 50 to 100γ daily for 3 weeks stimulates seminal vesicle growth much more than the same or even higher weights of androsterone. Wang and Wu (1933) also find the seminal vesicle test non-specific. Butenandt and his associates (1935) and the writer with David Duncan (unpublished) have employed the seminal vesicle weight reaction in normal immature rats as a test for the male hormone preparations. This is a fairly satisfactory method providing one avoids the use of the test when gonad-stimulating factors are present. In fact Zaharesco-Karaman, Proumbaru, and Fotin (1935) use the same principle in testing urine for pregnancy, hydatiform mole, or chorioepithelioma.

The influence of estrogenic substances on the seminal vesicle and prostate weight and structure. Freud (1933) was the first to report that theelin acts as a "pacemaker" for the male hormone in the seminal vesicle reaction. He pointed out how important it is to consider estrogenic impurities in applying this male hormone assay-method on concentrates and called attention to the specific action of the estrogenic substance in stimulating growth of the smooth musculature of the seminal vesicle. These observations have been confirmed by de Jongh (1934, 1935), Korenchevsky and Dennison (1934, 1936), and by Overholser and Nelson (1935). This estrogenic action is not eliminated by hypophysectomy (de Jongh) nor is it counteracted by progesterone (David, Freud, and de Jongh, 1934). David, Freud, and de Jongh (1934) also observed that in castrated mice the theelin produces stratified epithelium

at the orifice of the ducts from the prostate and seminal vesicles. The stratified epithelium is not produced by the pure male hormone but instead a spongy glandular structure. Heringa and de Jongh (1934) also observed that in young castrated mice, the theelin changes the connective tissue layer in the ampulla of the vas deferens from the fine collagen bundles and the reticulin network to a thick layer with increased intrafibrillar material which appears to be mucoid in character. These observations led Laqueur and de Jongh (1935) to consider prostatic hypertrophy as due to an improper balance of male and female hormones. Hence male hormone should counteract these actions. This de Jongh has demonstrated experimentally in part by increasing the dose of male hormone or decreasing the theelin dosage.

Other mammalian accessory sex-organ reactions to male hormone. The normal histology of the epithelium of the vas deferens and of Cowper's gland has also been regenerated in the castrated rat with male hormone concentrates by Vatna (1930) and Heller (1930, 1932) respectively. These reactions require 0.4 to 1.0 capon unit per day for 20 days as a minimum qualitative test and 6 to 7 units per day for complete restoration to the normal.

That the seminal vesicle and prostate of the castrate guinea pig can be maintained at a secretion level by male hormone concentrates has been shown by Moore and Gallagher (1930) and Kabak (1931) by the application of the Battelli electric ejaculation test (1922). The minimum daily dose necessary to produce a positive ejaculate is, however, of the order of 1.7 to 3 capon units.

The weight of the penis in the castrated rat has also been followed by the Korenchevsky group in parallel with their studies on the seminal vesicle and prostate. The rate of increase in weight with increasing doses follows a curve of the same character as those for seminal vesicle and prostate. The different androgenic substances act very differently on the different end organs, however. This will be covered in more detail later in this review.

The preputial glands of the castrated mouse have been regenerated by male hormone preparations by Voss (1931). In castrated rats, Dingemanse, Freud, and Laqueur (1935) have also shown an increase in the weights of the preputial and periurethral glands with male-hormone concentrates. Korenchevsky (1935) calls attention to the appearance of similar structures on the vagina at the base and in front of the bladder in ovariectomized rats on daily doses of 175 to 700 γ androstanediol-3,17. He reminds us of Virchow's statement that the periure-

thral glands found in the female are similar in structure to the male prostate.

Other biological reactions. Keck (1932, 1933, 1934) and Witschi (1936) have demonstrated that the bill pigmentation of the English sparrow is controlled by the male hormone. They have shown that the normal pigmentation can be restored in the bill of the castrated adult English sparrow in 10 to 20 days by the daily injection of 0.6 to 1.5 capon units. They suggest the method for quantitative purposes.

Glaser and Haempel (1931, 1932) suggest the production of the mating pigmentation in the castrated carp, *Cyprinus amarus*, or castrated *Rhodeus amarus* as the criterion for the male hormone. A fish unit is the minimum amount of substance necessary to produce the mating pigmentation in three out of four fish of the same weight in 6 to 7 hours after one intramuscular injection. André Beaume (1935) suggests using the non-castrated male fish, but it must not be in the spawning season and must be kept in water at 14 to 15°C. The fish are, however, rather susceptible to toxic substances found in urine extracts.

The quantitative extraction of the male hormone. The quantitative extraction of the male hormone from tissues, blood, and urine has grown in importance with every advance in our knowledge of the sex hormones. This is particularly true for urine. The historical development of this subject clearly illustrates how incompletely the first extractions of urine were carried out. Thus Loewe and Voss (1929) reported extracting sufficient hormone activity from 3.6 liters of men's urine to give a positive seminal vesicle regeneration test in the castrated mouse. Funk, Harrow, and Lejwa (1929, 1930) by chloroform extraction increased the yield of hormone per liter appreciably. Womack and Koch (1930) and Gallagher and Koch (1930) obtained still higher values in adult human male and female urines but no detectable amount in the urines from boys under 10 years of age or from bull calves. A systematic investigation on the completeness of extraction of urine in various types of continuous extractors and with various solvents has, however, shown that the content of human male urine is distinctly higher than the earlier studies indicate. Gallagher, Koch, and Dorfman (1935) recommended boiling the urine for two hours with the addition of one-tenth the volume of commercial hydrochloric acid and then extracting in a special extractor with at least ten volumes of benzene per volume boiled urine.

Although they found no difference in the yield of male hormone on extracting acidified unboiled urine and acidified urine boiled for two

hours respectively, recent unpublished observations by Gallagher, Peterson, and Koch (1936) show that a liberation and then a loss or destruction of androgenic material takes place within the two-hour period. The best yield has been obtained thus far by extracting the urine after boiling with hydrochloric acid for fifteen minutes.

The apparatus is based on the counter-flow principle and is so constructed that the benzene is very finely divided by being forced through a 40- to 80-mesh sintered glass filter before it comes in contact with the urine. By this procedure they obtain 18 to over 100 international units male hormone and 80 to 110 international estrogenic units per liter men's urine. Re-extraction of the urine yielded no further hormones. Gallagher, Koch, and Dorfman (1935) have also obtained practically the same yield of male hormone by adsorption on "dicalite," a commercial diatomaceous earth, and subsequent elusion by hot alcohol. The estrogenic material is not removed by dicalite.

Human urine appears to be unique with respect to male-hormone content, as is shown by the tabulation below:

	International units androgen
Human urine male (per 24 hours)	40–100
Human urine female (per 24 hours)	30–100
Stallion urine (per liter)	8
Bull urine (per liter)	<1
Ram urine (per liter)	4
Rat urine (per liter)	<1

The yield of hormone from bull testis-tissue still is exceedingly low. It varies from 50 to 150 grams fresh testis-tissue per international unit. All attempts to increase the yield by various methods of hydrolysis and by re-extraction have failed to improve the yield (Womack and Koch, 1930).

The extraction of the hormone from blood has been even less satisfactory. By applying the routine alcohol extraction to whole bull's blood, Gallagher (1928) could not detect it although he injected the equivalent of 200 cc. blood per day. Womack and Koch (1930) obtained comb growth on the equivalent of 300 to 600 cc. bull's blood. In view of these low values it is difficult to explain the results of other workers. Thus, Busquet (1927, 1928) reported that he produced crowing, treading, and comb growth in young and old capons by giving daily by mouth for 7 to 10 days, 5 cc. blood from bull, ram, or stallion. Blood from castrates gave negative results. Muto (1928) also reported that

he could retard the rate in weight decrease of the seminal vesicles in castrated mice by injecting the blood from the spermatic vein or the testicular lymph from a normal male mouse. Muto's evidence is of doubtful value because he has not allowed for animal variation and because the rate of regression of a seminal vesicle is an exceedingly poor criterion for hormone action. McCullagh, McCullagh, and Hicken (1933) report positive results on two capons by injecting the ether extract from 50 cc. oxalated human blood which had been dehydrated by 50 to 75 grams anhydrous sodium sulphate. They record similar results on human spinal fluid. Obviously careful quantitative studies are necessary in order to establish complete extraction methods on body fluids and tissues before we can hope to have our quantitative studies be of any value in diagnosis and in investigations on the distribution in normal and pathological conditions.

EXTRACTION AND PURIFICATION OF THE MALE SEX HORMONES, ANDROSTERONE AND DEHYDROISONANDROSTERONE, FROM URINE. The first natural source to yield a pure crystalline male hormone was human urine. Butenandt (1931, 1932) used as his starting material the raw oil obtained by chloroform extraction of acidified male human urine. An ether solution of this oil loses about 90 to 95 per cent by weight of inert material by shaking eight to ten times with 10 per cent sodium hydroxide. The neutral fraction left on evaporating the water-washed ether solution after repeated hydrolysis and subsequent partition between 50 to 70 per cent alcohol and petroleum ether leaves an alcohol-soluble oil which contains one Butenandt capon-unit per 300γ. By treating the latter with hydroxylamine, Butenandt obtained a crystalline product which no doubt was a mixture of oximes. The carefully purified oxime of melting point 215° contained approximately 1 Butenandt capon-unit per 100γ, but the comb-growth response was delayed and unusually prolonged. This indicates that the oxime itself is not active but that it is gradually hydrolyzed to the free active form in the organism. *In-vitro* hydrolysis did not yield the hormone in pure form directly. However, after careful sublimation at 80 to 85°C., at a pressure of 0.0001 mm. mercury, a substance was obtained of M.P. 178°, which Butenandt reported to contain 1 capon unit per 1 to 2γ. To this substance he then assigned the formula $C_{16}H_{26}O_2$; he considered it to be a saturated hydroxyketone. Later (1932) analysis of a substance of M.P. 160° led to the formula $C_{19}H_{30}O_2$ or $C_{18}H_{28}O_2$. On the basis of the empirical formula, $C_{19}H_{30}O_2$, and the chemical characteristics of the substance, he then suggested the structure now adopted for androsterone.

These conclusions were confirmed and supported by additional data by Butenandt and Tscherning (1934). The general procedure adopted by them for the preparation of androsterone is given below. The final product has, however, only an activity of 1 Butenandt capon-unit per 150 to 200γ whereas for his first crystals, Butenandt (1931) reported an activity of 1 to 2γ per capon unit. No one has been able to duplicate the production of so potent a substance nor have subsequent assays by Butenandt confirmed these earlier claims. It is very probable that the substances separated by Butenandt in 1931 and in 1934 are identical but that in the earlier assays on the 1931 product an unknown error was introduced.

A second male-hormone fraction was separated as a by-product from fraction 10aγ (p. 168) in 1934 by Butenandt and Dannenbaum. This substance ($C_{19}H_{27}OCl$) was found to be an ester-chloride derivative of dehydroisoandrosterone. It is very probable that the chloride is an artefact, obtained from the parent substance, dehydroisoandrosterone, which usually accompanies androsterone in men's urine. The chloride ester very likely is formed during the extraction of the acidified urine by boiling with chloroform. The relation of the dehydroisoandrosterone and chloride ester thereof to androsterone was proved by Butenandt and his associates (1934, 1935). In the later (1935) studies, they concluded that men's urine contains practically equal amounts of androsterone and dehydroisoandrosterone. This statement is based on the use of digitonine, which precipitates the dehydroisoandrosterone, but not the androsterone. The yield of the two substances in pure form is approximately 20 mgm. of each per 100 liters of urine. The actual amount present probably is more nearly 20 to 40 times that quantity.

Butenandt and Tscherning method for the preparation of androsterone from men's urine

Men's urine (1 capon unit* = 200 cc.)
↓ Concentrated, acidified, and extracted with $CHCl_3$
Chloroform extract (1 capon unit = 150 to 160 mgm.)
↓ Extracted with KOH → Alkali soluble (90 to 95%)
Neutral, undissolved part (1 capon unit = 9 to 10 mgm.)
↓ Distillation with steam → Volatile fraction (30 to 35%)
Non-volatile fraction (1 capon unit = 6 mgm.)
↓ Alkaline hydrolysis → Saponifiable fraction (45%)

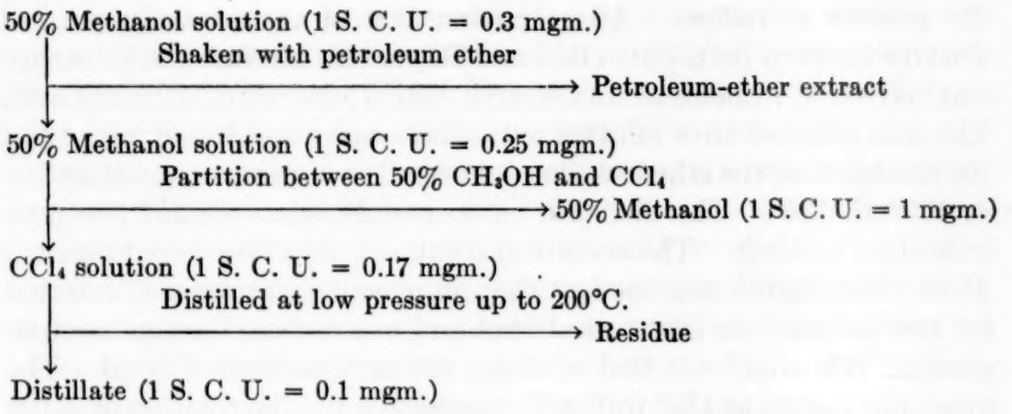
Non-saponifiable fraction I (1 capon unit = 3 to 4 mgm.)
↓ Acid hydrolysis → Hydrolyzed material (15 to 20%)
Non-saponifiable fraction II (1 capon unit = 2.8 to 3 mgm.)
↓ Partition between benzene and petroleum ether → Benzene soluble 10a, α (12%)
Petroleum-ether soluble
↓ Extraction by 60% alcohol → Petroleum-ether soluble fraction 10a, γ (51%) (This used for the chlorketone)
60% Alcohol-soluble 10a β (1 capon unit = 1 to 1.4 mgm.)
↓ Oxime formation
Oxime of M.P. 215 to 221°
↓ Hydrolysis by dilute acid
Androsterone, $C_{19}H_{30}O_2$, M.P. 178° (1 capon unit = 150 to 200γ)

* Butenandt capon unit ≏ 150 to 200γ androsterone or 1.5 to 2.0 I. U.

Gallagher and Koch (1934) also prepared concentrates from men's urine, but did not obtain the pure substances in crystalline form. However, it is interesting to note that the potency, 100γ per international unit, of the final concentrate (see flow sheet, below) was of the same order as that of androsterone.

Gallagher and Koch fractionation procedure of the male-hormone activity from men's urine

Men's urine
↓ Acidified and boiled with 10% (by volume) commercial HCl. Extracted with benzene in a continuous extractor.
Benzene extract (1 S. C. U.* = 10 mgm.)
↓ Evaporated, dissolved in ether, and extracted with 10% NaOH → NaOH
Ether extract (1 S. C. U. = 1 mgm.)
↓ Distilled at low pressure at 150°C. → Distillate
Residue (1 S. C. U. = 0.5 mgm.)
↓ Solution in 70% ethanol → "Tar"
70% Ethanol solution (1 S. C. U. = 0.4 mgm.)
↓ Evaporated, dissolved in 50% methanol → "Tar"


50% Methanol solution (1 S. C. U. = 0.3 mgm.)
Shaken with petroleum ether
Petroleum-ether extract
50% Methanol solution (1 S. C. U. = 0.25 mgm.)
Partition between 50% CH3OH and CCl4
50% Methanol (1 S. C. U. = 1 mgm.)
CCl4 solution (1 S. C. U. = 0.17 mgm.)
Distilled at low pressure up to 200°C.
Residue
Distillate (1 S. C. U. = 0.1 mgm.)

* S. C. U. = standard capon unit = 150 to 160γ androsterone or 1.5 to 1.6 international units.

EXTRACTION AND PURIFICATION OF THE MALE HORMONE, TESTOSTERONE, FROM TESTIS TISSUE. The McGee (1927), Gallagher and Koch (1929, 1934) process carried the activity of the concentrate from bull testis-tissue to approximately ten times that of the urine concentrates. This clearly indicated that the male-hormone activity obtained from these two sources is due to different substances. The method employed is to extract the finely ground tissue at room temperature with four volumes by weight of 95 per cent alcohol for three to five days. After concentrating the alcoholic extract under diminished pressure to an aqueous sludge, it is thoroughly extracted with benzene. The benzene extract is evaporated to dryness and the residue dissolved in acetone. The acetone solution is chilled at -10°C. for 24 hours and filtered. After the acetone is removed by evaporation, the solids are dissolved in hexane and thoroughly shaken with 70 per cent alcohol. The alcohol is removed by evaporation, the solids dissolved in ether and this solution shaken with 10 per cent aqueous NaOH. Further purification by the use of immiscible solvents similar to those employed in the fractionation of the urine extract yielded products approximately ten times as potent as androsterone by the comb-growth assay method.

Dingemanse, Freud, Kober, Laqueur, and Münch (1930, 1931) also fractionated the bull testis-tissue extracts by means of solvents to a stage where 0.05 to 0.10 mgm. contained the potency of one of their capon units. This material, when submitted to high vacuum distillation at 80 to 112°C. yielded a particularly active fraction at 90°C. and 0.001 mm. mercury pressure. The product was not in pure crystalline form. In 1935, David, Dingemanse, Freud, and Laqueur announced the separation of crystals of exceptional potency. David (1935) describes

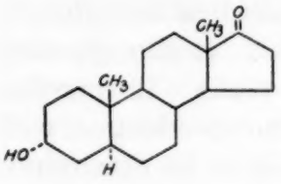
the process as follows. After fractionation of the partially purified activity between petroleum ether and 70 per cent alcohol, the substance was recovered in benzene and shaken with 75 per cent sulphuric acid. The acid solution after dilution with water was extracted by ether and the residue from the ether solution submitted to high vacuum distillation at 110° to 130°. The distillate on recrystallization yielded the pure crystalline product. The acetate and oxime derivatives were prepared. These observations and the fact that no phenol character was detected led him to conclude that one alcohol and one carbonyl group must be present. He could not find evidence for an unsaturated bond. The substance melted at 154° to 154.5°, possessed a specific rotation of +109 and analyzed $C_{19}H_{28}O_2$. The activity was reported as 7 to 10 times that of androsterone by the capon test. It is the only crystalline hormone of high potency which has been separated from testis tissue.

Frattini and Maino (1930, 1932, 1935) reported the separation of the testis hormone in crystalline form a number of times. They have never given conclusive evidence that they have actually separated a pure substance. Furthermore, the procedures, yields, properties, and potency of their product do not inspire confidence in their claims. In their first publication they consider 3000 grams of fresh tissue to be equivalent to 3 mgm. of their product and claim that 3 mgm. is a capon unit, that it possesses estrogenic action, that it is water soluble, and exists as a sodium salt. In 1932 and 1935, they state that 500γ of their product in ten days produces 40 to 50 per cent increase in the comb length. They do not give any analytical data on composition or on the formation of derivatives of their substance.

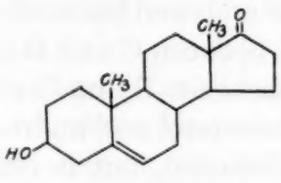
Ogata and Hirano (1933, 1934) also claim to have separated the male hormone in pure form from hog testicles. They report that the non-saponifiable fraction remaining after the saponification of a 70 per cent alcoholic extract with $Ba(OH)_2$ when subsequently purified by resolution in absolute alcohol and then treated with hot hexane in ether solution yielded a deposit on cooling which in doses of 480γ constituted a capon unit. In their second report they also saponify boar testes with 5 per cent aqueous KOH for several hours and extract with ether. To remove cholesterol, the usual treatment with acetone and hexane was applied. After separating inert materials from 70 per cent alcohol and acetic-ether solutions, the soluble fraction in absolute alcohol was precipitated by hexane, then redissolved in 50 per cent ethyl alcohol and allowed "to evaporate for a few days in a cool place." The material

which separated was recrystallized from 50 per cent ethyl alcohol. It had a melting point of 128 to 130° and an activity of 1 capon unit per 850γ given daily for two days. The yield was 5 mgm. from 42 kgm. of tissue. No data on composition or derivatives were given.

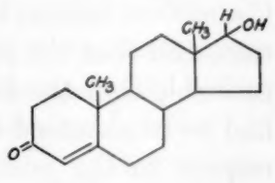
THE PROOF OF THE STRUCTURE OF ANDROSTERONE, DEHYDROISOANDROSTERONE, AND TESTOSTERONE AND THE PREPARATION OF VARIOUS RELATED COMPOUNDS BY DEGRADATION STUDIES ON STEROLS AND BILE ACIDS. The chemical investigations on androsterone, dehydroisoandrosterone, and testosterone as prepared from urine and testis tissue respectively gave the empirical formulae, $C_{19}H_{30}O_2$, $C_{19}H_{28}O_2$, and $C_{19}H_{28}O_2$ respectively and suggested these structural formulae:



ANDROSTERONE



DEHYDROANDROSTERONE

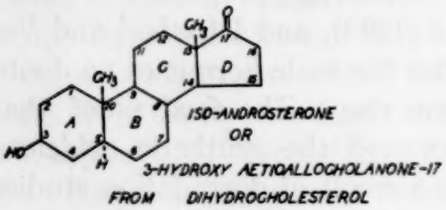


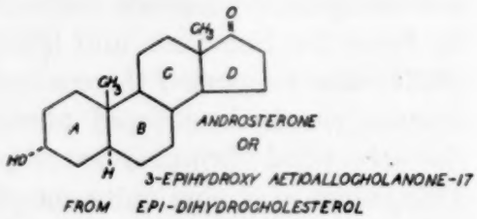
TESTOSTERONE

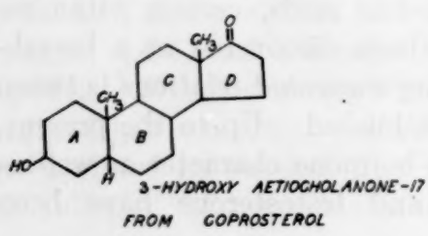
The relations of androsterone to dehydroisoandrosterone and to the chlorketone were clearly shown by Butenandt and Dannenbaum (1934) and Butenandt, Dannenbaum, Hanisch, and Kudszus (1935). Furthermore, the fact that pure theelin (oestrone), $C_{18}H_{22}O_2$, was converted into an octa-hydrogen derivative by intensive hydrogenation and that this new compound possesses comb-growth stimulating properties as shown by Schoeller, Schwenk, and Hildebrand (1933), and Dirscherl and Vos (1934) also supported the conclusion that the male hormones no doubt contain the hydrogenated phenanthrene ring. The final proof that the structural formulae are correct awaited the synthetic evidence. This evidence is now quite complete as a result of degradation studies on sterols and bile acids. Incidentally the investigations on sex hormones and vitamin Ds have stimulated the production, characterization, and degradation of sterols so intensively that new and remarkable structural relations among the sterols, bile acids, certain vitamins, hormones, and cardiac glucosides have been discovered at a breath-taking pace and for the first time the long suspected relations between certain of these groups are now well established. Up to the present, twenty-seven related substances of male-hormone character as well as androsterone, dehydroisoandrosterone, and testosterone have been

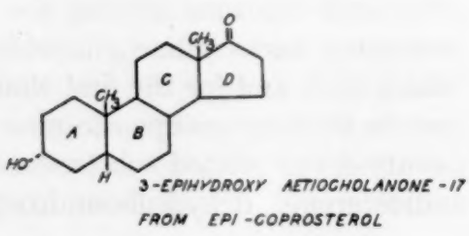
prepared by the synthetic degradation of sterols. The structural relations of these to each other are shown on pp. 180–189 and a more detailed chemical discussion of each is given below.

(cis)-*Androsterone* (3-*epi*-hydroxy-aetio-*allo*cholanone-17) was first prepared from cholesterol by Ruzicka, Goldberg, Meyer, Brüngger, and Eichenberger (1934, 1935). Theoretically it would appear a relatively simple matter to protect the alcohol group in position 3 by acetylation, to hydrogenate the 5,6 double bond, and then to destroy the side chain in position 17 by oxidation. However, the control of the oxidation, the further complication of stereo-isomerism on carbon 5 as well as the presence of six other asymmetric carbon atoms in the cholane ring presented numerous difficulties. It was already known that rings C and D in various known sterols and bile acids are identical in configuration, and that the junction between C and D is *trans*. It also appeared probable that the linkage between B and C also is trans. It therefore had to be assumed that cholesterol and androsterone are identical with respect to the positions discussed, but it remained to be determined whether after the saturation of position 5,6, we require the dihydrocholesterol or coprosterol ring. Finally it was a surprise to learn that even the asymmetry of carbon atom 3 was involved and that androsterone is a derivative of the *epi* form of dihydrocholesterol and not of coprosterol. These relations were pointed out and solved by Ruzicka et al. (1934, 1935). The structural relations and the terminology are reproduced below:









The steps involved in the preparation of androsterone by Ruzicka et al. (1934) are *a*, the hydrogenation of cholesterol to dihydrocholesterol; *b*, the oxidation of the dihydrocholesterol to cholestanone by chromic acid; *c*, reduction of the cholestanone to a mixture of the two forms of dihydrocholesterol; *d*, separation of the *epi*dihydrocholesterol from the reduction mixture; *e*, conversion into the acetate to protect the alcohol group; *f*, oxidation of the side chain; *g*, separation of androsterone acetate as the semicarbazone; *h*, hydrolysis of the semicarbazone and crystallization of the androsterone.

Butenandt, Dannenbaum, Hanisch, and Kudszus (1935) proved the preparation of androsterone from cholesterol with dehydroandrosterone as an intermediate. The steps involved were *a*, cholesterol acetate is brominated and then oxidized by chromic acid to form the dibromketone; *b*, removal of bromine by zinc dust to form dehydroandrosterone; *c*, separation of the semicarbazone of dehydroandrosterone; *d*, hydrolysis; *e*, conversion of the dehydroandrosterone into the 3-chlorketone by treatment with thionyl chloride or with alcoholic HCl. This chlorketone was identical with the chlorketone Butenandt and Dannenbaum (1934) previously separated from urine and which they converted into androsterone acetate by hydrogenation and subsequent vigorous treatment with potassium acetate in glacial acetic acid. This treatment not only removes the chlorine in position 3 but also forms the epimer form of androsterone acetate. In other words, the original "dehydroandrosterone" is really the 5,6 unsaturated iso-androsterone.

Dalmer, Werder, Honigmann, and Heyns (1935) proved the relation of androsterone to the natural bile-acid group, which is, that the OH group in position 3 is the *epi* form in both cases, but that in position 5 the natural bile acids and coprosterol are identical, whereas cholesterol, sitosterol, stigmasterol, and androsterone yield or are of the opposite configuration or *allo* form. They accomplished this *a*, by preparing 3-hydroxy-*allo*cholanic acid from hyodesoxycholic acid obtained from hog bile; *b*, by repeated alternate treatments thereof with Grignard reagent, acetylation, and oxidation until the C_{17} side chain was removed step by step and replaced by the ketone oxygen as in androsterone. These investigators also prepared the same 3-hydroxy-*allo*cholanic acid from the two hydrogenated sterols obtained from sitosterol ($C_{29}H_{50}O$) and stigmasterol ($C_{29}H_{48}O$).

Dirscherl (1935) used cinchol ($C_{29}H_{50}O$), a sterol obtained from cinchona bark as the starting material. After hydrogenation he carried the dihydrocinchol through essentially the same steps first used so successfully by Ruzicka et al. (1934) and obtained androsterone.

Marker (1935) and Marker, Whitmore, Kamm, Oakwood, and Blatterman (1936) carried out the preparation of androsterone through the following steps: *a*, treatment of cholesterol with PCl_5 to form cholesteryl chloride; *b*, conversion into 3-chlor-5,6-dibromcholestane; *c*, oxidation by chromic acid and debromination to form 3-chlor-Δ^5-androstenone (3-chlor-Δ^5-dehydroisoandrosterone); *d*, hydrogenation of the latter to form 3-chlorisoandrosterone; *e*, the hydrolysis of this yielded androsterone. These investigators and Ruzicka (1934) avoided the bromination step by *a*, hydrogenating the cholesteryl chloride to form cholestyl chloride, or *b*, by forming dihydrocholesterol before introducing the chloride. The cholestyl chlorides formed by the two latter methods did not agree in melting points nor did the chlorandrosterones which were obtained by chromic-acid oxidation agree in melting points. However, the chlorandrosterone of Marker, et al., on hydrolysis with potassium acetate yielded androsterone. It appears then that in the vigorous treatment with potassium acetate we have a Walden rearrangement from the isoandrosterone to the form of androsterone which occurs in urine.

Isoandrosterone (3-hydroxy-aetio*allo*cholanone-17) has also been prepared from cholesterol by Ruzicka et al. (1934) and from stigmasterol by Butenandt and Cobler (1935). The former group followed essentially the same methods as for androsterone from dihydrocholesterol only it was not necessary to shift the position of the OH and H on carbon atom 3. Butenandt and Cobler (1935) formed as an intermediate oxidation product *allo*pregnanolonone which is of interest because it is obtained from progesterone by hydrogenation and reduction of the 3-keto group. By the use of Grignard reagent and chromic-acid oxidation, the *allo*pregnanolone was converted into isoandrosterone. The *allo*-pregnanolone has also been separated from corpus-luteum extracts. Butenandt, Tscherning, and Hanisch (1935), and independently Wallis and Fernholz (1935) also prepared the isoandrosterone by the catalytic hydrogenation of dehydroisoandrosterone obtained from cholesterol.

The other two isomers theoretically possible from cholesterol are the 3-*epi*hydroxyaetiocholanone-17 and 3-hydroxyaetiocholanone-17. These are more closely related to coprosterol and were also prepared by Ruzicka et al. (1934). They were not found active in the capon in doses of 1000γ.

Androstanol-17-one-3 (17-hydroxyaetio*allo*cholanone-3) is obviously another isomer of androsterone with an exchange in the OH and O positions. Butenandt, Tscherning, and Hanisch (1935) prepared this by oxidizing isoandrostanediol-monoacetate-17 with chromic acid and

also by the hydrogenation of testosterone. The high activity, 25γ per capon unit, is of interest because of the close relationship to testosterone.

Dehydroisoandrosterone (Δ^5-3-hydroxy-aetio-cholenone-17 or Δ^5-isoandrostenol-3-one-17), as previously stated was first separated from urine by Butenandt, Dannenbaum, Hanisch, and Kudszus (1934, 1935). They also prepared it from the 3-chlorketone-17 derivative separated as an artefact in the fractionation of urine extracts and finally they prepared it from cholesterol and stigmasterol by the following steps: *a*, formation of dibromide of cholesterol acetate; *b*, oxidation by chromic acid to form the acetate of isoandrosteronedibromide; *c*, debromination by zinc dust in acetic acid; *d*, separation of the dehydroisoandrosterone semicarbazone; *e*, hydrolysis of the semicarbazone derivative to free dehydroisoandrosterone. Oppenauer (1935) also prepared the substance by essentially the same methods from γ-sitosterol. Ruzicka and Wettstein (1935) and Wallis and Fernholz (1935) carried out practically the same procedure as that of Butenandt in the preparation of the substance from cholesterol.

It is now generally agreed that the dehydroandrosterone obtained from urine is identical with the synthetic degradation product described above. This leads to the conclusion that dehydroisoandrosterone is the better name and that carbon 3 has the same configuration in cholesterol, stigmasterol, isoandrosterone, and dehydroisoandrosterone. This is further confirmed by the fact that dehydroisoandrosterone is precipitated by digitonin whereas the 3-*epi* compounds, such as bile acids and androsterone are not precipitated (Schoeller, Serini and Gehrke, 1935). In fact the Butenandt group (1935) makes use of this reaction in the separation of the dehydro form from urine.

Ruzicka, Fischer, and Meyer (1935) have also been able to shift the double bond in Δ^5-trans-dehydroandrosterone to the 4-5 position by the usual treatment with hydrochloric acid dissolved in absolute alcohol thus producing Δ^4-trans-dehydroandrosterone.

Androstanediol-3-cis-17-trans (dihydroandrosterone or 3-*epi*-hydroxy-*allo*-aetiocholanol-17-trans). After Schwenk and Hildebrandt (1933) and Girard, Sandulesco, and Fridenson (1933) had prepared oestradiol (dihydro-oestrone or dihydrotheelin) from theelin by reduction of the ketone group and found the product to possess greater oestrogenic potency it is not surprising that similar reduction studies were carried out on androsterone. Butenandt and Tscherning (1935) reduced androsterone to the diol by sodium in propyl alcohol. Ruzicka, Goldberg, and Meyer (1935) carried out the reduction by hydrogen in presence of

platinum oxide in acid and alkaline solutions. From the table, pp. 180–189 it is obvious that this diol also is more active than the ketone from which it is obtained.

However, by the reduction of the ketone-17 group to a secondary alcohol we have introduced another asymmetric carbon atom. As a result we now have possible four different androstanediols-3-17. The form discussed above has been assumed to be the 3-cis-17-trans form, that is position 3 is as in androsterone and in position 17, the OH group is considered to be trans to the CH_3 group on carbon 13. Obviously the 17-trans reduction product of isoandrosterone, that is, androstanediol-3-trans-17-trans is also possible. It has in fact been prepared by reduction of isoandrosterone by Butenandt, Tscherning and Hanisch (1935) and by Ruzicka, Goldberg, and Rosenberg (1935). Still another isomer, the 3-trans-17-cis diol, has also been prepared by Ruzicka and Goldberg (1936) from isoandrosterone and from androstenediol-3-trans-17-cis. The 3-cis-17-cis diol does not appear to have been identified as yet although it may easily be present in some of the products of reduction of androsterone. The comparative biological assays on these different androstanediols indicate that in position 3 the cis form raises the potency whereas in the 17 position it appears that there is very little difference between the potencies of the cis and trans forms (Ruzicka and Rosenberg, 1935, 1936).

17-Methylandrostanediol (17-methyl-3-*epi*hydroxy-*allo*-aetio-cholanol-17). This substance was obtained in good yield by treatment of androsterone with methyl magnesium iodide by Ruzicka, Goldberg and Meyer (1935). It is of about the same order of activity as androstanediol in the capon, that is, about three times as potent as androsterone. The configuration of carbon 17 has not been determined.

17-Methylandrostanol-17-one-3 (17-methyl-3-keto-*allo*-aetio-cholanol-17). By first preparing the 17-methylandrostanediol from androsterone from the action of the methyl magnesium-iodide reagent and then oxidizing cautiously with chromic acid, the alcohol in position 3 was converted to a ketone group (Ruzicka, Goldberg, and Rosenberg, 1935). This substance is approximately six times as active as androsterone in the capon test.

3,17-Dimethylandrostanediol-3,17 (3,17-dimethyl-*allo*-aetio-cholanediol-3,17). This substance was obtained by the interaction of methyl magnesium iodide with the diketone, androstanedione-3,17. The very low potency indicates that methylation at position 3 lowers the activity of the saturated compound (Ruzicka, Goldberg, and Meyer, 1935).

17-Ethyl-cis,androstanediol-3,17 (17-ethyl-3-*epi*hydroxy-*allo*-aetio-cholanol-17). This was prepared in the same way by ethyl magnesium iodide with cisandrosterone. This product is approximately five times as active as androsterone in the capon test (Ruzicka, Goldberg and Meyer, 1935).

17-Ethyl-trans-androstanediol-3,17 (17-ethyl-*allo*-aetio-cholanediol-3-trans, 17). By the same reaction applied to the transandrosterone, Ruzicka, Goldberg, and Meyer (1935) prepared this trans-isomer of exceedingly low activity. This again emphasizes the importance of the configuration of carbon atom 3.

17-Ethyl-androstanol-17-one-3 (17-ethyl-3-keto-*allo*-aetio-cholanol-17). The same investigators prepared this substance by the cautious oxidation of 17-ethyl-trans-androstanediol-3,17 with chromic acid. Its activity has not been reported, but it probably is of high order of activity.

Androstanedion-3,17 (*allo*-aetio-cholanedione-3,17). This product was prepared by Butenandt and Tscherning (1934) and Ruzicka, Goldberg, and Meyer (1935) from androsterone by mild oxidation with chromic acid. It has been prepared similarly from androstanolone (Butenandt, Tscherning, and Hanisch, 1935), isoandrosterone (Butenandt and Cobler, 1935), and androstanediol (Ruzicka and Rosenberg, 1936). It is approximately one-half as active as androsterone in the capon.

Testosterone (Δ^4-androstenol-17-one-3 or Δ^4-3-keto-aetio-cholenol-17). Testosterone was first separated in crystalline form from bull testis-tissue by Laqueur et al. (1935). The chemical characteristics are very different from those of androsterone. This was not surprising because numerous studies on concentrates from testis tissue and urine respectively convinced many of us that the hormones from the two sources are not identical. It was first suggested chemically by the destruction of the testicular product by basic hydrolysis (McGee, Juhn and Dommn, 1928; Gallagher and Koch, 1933, 1934). Biological differences were first clearly proved by Laqueur de Fremery, Freud, de Jongh, Köber, Luchs and Münch (1931) who found 1400 per cent variations in ratios of capon assays to rat seminal-vesicle and prostate reactions on different urinary and testis-tissue concentrations and different fractions of each. This has been repeatedly confirmed by Freud and Laqueur (1934), Freud (1934), Dingemanse, Freud, and Laqueur (1935), David and Freud (1935), and by Callow and Deanesly (1935). Matsuzaki (1933) and Ogata and Hirano (1933, 1934) also reported that treatment of an acetone solution of the testis-tissue

concentrate with potassium permanganate causes destruction of the activity on rat seminal vesicles but not on the capon. They could not confirm the lability of the comb-growth stimulating factor to alkaline hydrolysis, but reported loss of seminal-vesicle stimulation as a result of such treatment. Many of these observations on urine and testis-tissue concentrates have been clarified as a result of the excellent biological results obtained from the various synthetic degradation products already discussed, but the artificial preparation of testosterone and various derivatives thereof by synthetic degradation methods, very soon after its first separation from testis-tissue concentrates, gave the best answer.

It may be recalled that trans-dehydroandrosterone was found to possess very low potency, that when it was saturated and oxidized to androstanedione, the activity was slightly improved, but that when the 17 position was reduced to form androstanol-17-one-3, a very active product was formed. Furthermore, reduction of androsterone to the androstanediol also increased the potency. The instability of the testis-tissue concentrates in alkaline solutions and the similar behaviour of progesterone also suggested that possibly the real male hormone as found in the tissues is a derivative of Δ^4-aetio-cholone-3. Stimulated by these findings, the studies on the artificial preparation of derivatives of the unsaturated (cholene or androstene) series were already under way at the time the Laqueur group first announced the isolation of testosterone from testis tissue. At practically the same time, Butenandt and Hanisch (1935) and Ruzicka and Wettstein (1935) announced the artificial preparation of testosterone, identical in physical, chemical, and biological properties with natural form. The process involved the following steps: *a*, reduction of Δ^5-dehydroandrosterone to Δ^5-androstenediol; *b*, preparation of the 3,17-diacetate of the diol; *c*, partial hydrolysis to form the 17-mono-acetate-androstenediol; *d*, formation of the dibromide in order to protect the double bond; *e*, oxidation by chromic acid in the cold and debromination by zinc dust or sodium iodide to form Δ^4-androstenol-17-one-3-17-acetate; *f*, mild saponification to form the free testosterone. Ruzicka, Wettstein, and Kägi (1935) later carried out the same steps beginning with Δ^5-androstenediol-3-acetate-17-benzoate. The maximum absorption spectrum of the substance at 238 mμ is correlated with the location of the unsaturated bond in the 4–5 position. The relation to cholestenone and progesterone is thus confirmed. The further relation of testosterone to an isomer of androsterone was shown by hydrogenating testosterone to form androstanol-17-one-3 (Butenandt, Tscherning, and Hanisch, 1935).

Δ^5-Androstenediol-3-trans-17-cis (Δ^5-aetio-cholenediol-3,17). Ruzicka and Wettstein (1935) and Butenandt and Hanisch (1935) prepared this substance from Δ^5-dehydroandrosterone by reduction with sodium in propyl alcohol or nickel catalyst in alcoholic solution.

Δ^4-Androstenedione-3,17 (Δ^4-aetio-cholenedione-3,17). Ruzicka and Wettstein (1935) and Butenandt and Kudszus (1935) prepared this by *a*, brominating dehydroandrosterone; *b*, oxidizing by chromic acid in acetic acid to form the diketone-dibromide; *c*, debrominating in acetic acid by zinc. Mild oxidation of testosterone also produced the androstenedione thus confirming the structural relations. In this connection the very recent observations by Butenandt and Dannenberg (1936) on Δ^1-androstenedione and Butenandt and Riegel (1936) on 6-oxytestosterone are of interest because they find both of the products to be practically free from androgenic action but to possess some estrogenic properties. When we recall that Δ^4-androstenedione is a very potent androgen, this result on the Δ^1-androstenedione is very remarkable.

17-Methyl-testosterone (Δ^4-17-methyl-androstenol-17-one-3). This was prepared by Ruzicka, Goldberg, and Rosenberg (1935) by *a*, treatment of dehydroandrosterone with methyl magnesium iodide; *b*, bromination; *c*, mild oxidation by chromic acid for 20 hours; *d*, separation of the dibromide as such; *e*, debromination by zinc, and *f*, sublimation.

17-Ethyl-testosterone (Δ^4-17-ethyl-androstenol-17-one-3). Ruzicka and Rosenberg (1936) and Butenandt, Cobler, and Schmidt (1936) proceeded as follows: *a*, prepared Δ^5-17-ethyl-androstenediol; *b*, brominated to the dibromide; *c*, oxidized at room temperature by chromic acid, and *d*, debrominated by zinc.

Δ^5-17-Methylandrostenediol-3,17 (Δ^5-17-methylaetiocholenediol-3,17 and Δ^5-17-ethylandrostenediol-3,17 (Δ^5-17-ethylaetiocholenediol-3,17). Ruzicka, Goldberg and Rosenberg (1935) prepared these substances by treatment of dehydroandrostrone in ether solution by the Grignard reaction. The ethyl derivative was also prepared by Butenandt, Cobler, and Schmidt (1936).

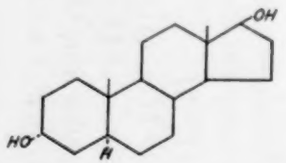
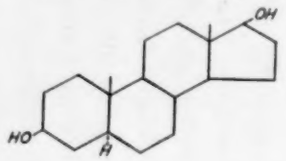
Androstanol-3-amine-17 (17-amino-3-*epi*hydroxy-*allo*-aetiocholane) and Δ^5-androstenol-3-amine-17 (Δ^5-17-amino-3-hydroxy-aetiocholene) were prepared by Ruzicka and Goldberg (1936) by reducing the oximes of androsterone and transdehydroandrosterone respectively in alcoholic solution with metallic sodium.

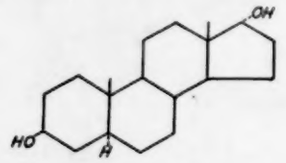
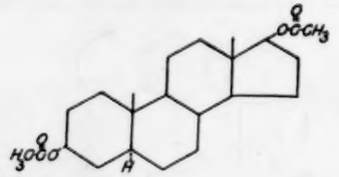
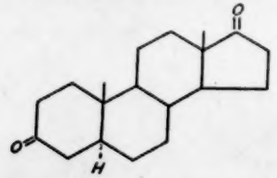
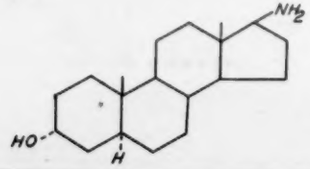
THE RELATIVE ACTIVITIES OF THE VARIOUS CONCENTRATES AND OF PURE PREPARATIONS. Table 2 on pages 180 to 189 lists in alphabetical order the names, structural formulae, and physical constants of 32

TABLE 2

Alphabetically arranged list of androgens

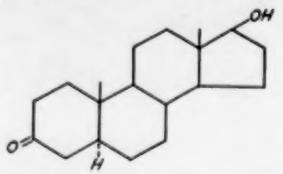
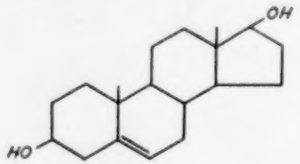
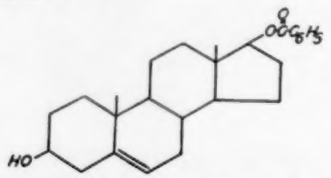
In the second column the initials of authorities quoted on activity are given above the horizontal division and below are given the sources from which the substances have been prepared. In the third column, the horizontal division separates the melting point and specific rotation values.

NAMES FORMULAE	AUTHORITY* Found in or prepared from	M.P. [α]	PHYSIOLOGICAL ACTIVITY					
			Capon test		Order of potency on rats			
					On minimum weight basis		On capon unit basis	
			γ Equiv. to 100 γ androsterone	Order of potency	Prostate	S. V.	Prostate	S. V.
Androstanediol-3-cis-17	B. & T.	221–223°	25	2	—	—	—	—
or Dihydroandrosterone	T. & R.		23	2	4	5	6	1
or 3-epi-hydroxy-aetio-*allo*cholanol-17	D. & P.		33	2	4	5	7	5
	Androsterone	+12.6° at 23°						
Androstanediol-3-trans-17-trans	B. & T.	164–168°	600	6				
or Isodihydroandrosterone	T. & R.		520	5				
	Isoandrosterone	+4.2° at 20°						

Androstanediol-3-trans-17-cis or 3-hydroxy-aetio*allo*cholanol-17-cis 	═══ Isoandrosterone and androstenediol	178–179° ═══						
Androstanediol diacetate or Dihydroandrosterone diacetate 	B. & T. T. & R. ═══ +12.5° at 23°	159–160° 162–163° ═══	25–35 (delayed response) 25–30	2				
Androstanedione-3,17 or aetio-*allo*cholanedione-3,17 	B. & T. T. & R. ═══ Androsterone, iso-androsterone, androstanediol, or androstanolone-17,3	129° 132–133° ═══ +104.8° at 20°C.	125 120	4	— 7	— 7	— 7	— 5
Androstanol-3-amine-17 or 17-amino-3-epihydroxyaetio-*allo*-cholane 	T. & R. ═══ Androsteroneoxime	187–188°	>1000	8	>11	>11	—	—

* B. & T. = Butenandt and Tscherning, T. & R. = Tschopp and Ruzicka, D. & P. Deanesly and Parkes.

TABLE 2—*Continued*

NAMES FORMULAE	AUTHORITY* Found in or prepared from	M.P. [α]	PHYSIOLOGICAL ACTIVITY: Capon test γ Equiv. to 100 γ androsterone	Capon test Order of potency	Order of potency on rats: On minimum weight basis Prostate	On minimum weight basis S. V.	On capon unit basis Prostate	On capon unit basis S. V.
Androstanol-17-one-3 or 3-keto-aetio-*allo*cholanol-17 	B. & T. Androstanediolmono-acetate, or dehydroandrosterone	178–180° +32.4° at 20°	25–30	2				
Δ⁵-Androstenediol-3-trans-17-cis or Δ⁵-aetiocholenediol-3-trans-17-cis 	B. & T. T. & R. D. & P. Transdehydroandrosterone	173–173.5° 175–178° 182–183° −55.5° at 18°	650 525 235	7 5 6	— 6 7	— 10 7	— 3	— 1
Δ⁵-Transandrostenediol-3,17-benzoate-17 or Δ⁵-transaetiocholenediol-3,17-benzoate-17 	T. & R.	220.5–222°			6	10	—	—

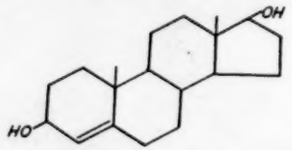
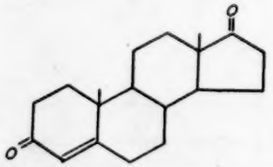
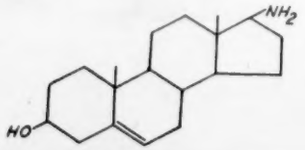
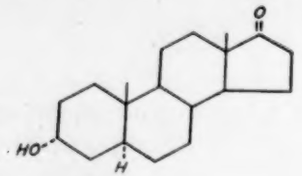
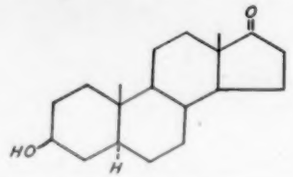
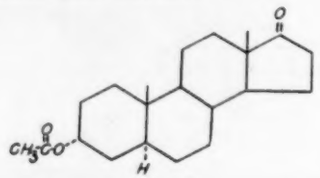
Δ^4-Androstenediol-3-trans-17 or Δ^4-aetiocholenediol-3-trans-17 	B. & T. ═══ Testosterone	128.5–130° ═══	>150(?)					
Δ^5-Transandrostenediol diacetate	T. & R.	165–166° 159.5° ═══ −56.5° at 18°	500	5				
Δ^5-Transandrostenediol monoacetate		147–148° ═══ −62.4° at 18°						
Δ^4-Androstenedione-3,17 or Δ^4-aetiocholenedione-3,17 	B. & T. T. & R. D. & P. ═══ Transdehydroandrosterone or testosterone	169° ═══ +185° at 18°	100 120 100		— 5 6	— 4 6	— 2 4	— 2 4
Δ^5-Androstenol-3-amine-17 or Δ^5-17-amino-3-hydroxy-aetiocholene 	T. & R. ═══ Δ^5-transdehydroandrosterone oxime	160–162° ═══	>1000	8	>11	>11		

TABLE 2—*Continued*

NAMES FORMULAE	AUTHORITY* Found in or prepared from	M.P. [α]	PHYSIOLOGICAL ACTIVITY: Capon test γ Equiv. to 100 γ androsterone	Capon test Order of potency	Order of potency on rats: On minimum weight basis Prostate	On minimum weight basis S. V.	On capon unit basis Prostate	On capon unit basis S. V.
(cis)-Androsterone or 3-epi-hydroxy-aetio-*allo*cholanone-17 	B. & T. T. & R. D. & P. Human urine, sterols, bile acids	182–183° +94.5° at 18°	100 100 100	3 4 4	— 8 8	— 8 8	— 8 8	— 6 9
(trans)-Androsterone or isoandrosterone or 3-hydroxy-aetio-*allo*-cholanone-17 	B. & T. T. & R. D. & P. Sterols	174–175°	700 770 700	8 7 7				
Androsterone acetate 	B. & T. T. & R.	160–161° +86.14° at 20°	100 (delayed) 100	4				

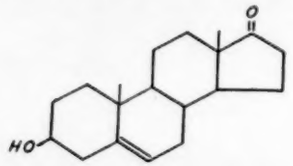
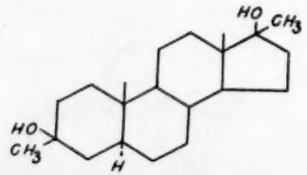
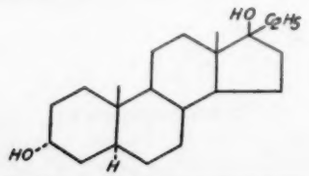
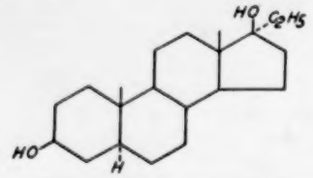
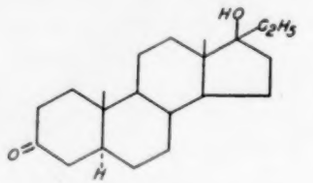
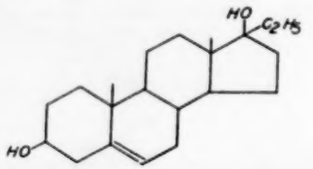
Androsterone benzoate		178–178.5°						
Androsterone succinate	T. & R.	185–185.5°	110	4				
Dehydroisoandrosterone or Δ^5-3-hydroxy-aetio-cholenone-17 or Δ^5-androstenol-3-trans-one-17 	B. & T. T. & R. D. & P. ═══ Human urine, sterols	137–138° and 148° (two forms) ═══ +10.9° at 18°	300 250 300	5 4 to 5 5 to 6	— — 9	— — 9	— — 10	— — 10
3,17-Dimethylandrostanediol-3,17 or 3,17-dimethylaetio-*allo*cholane-diol-3,17 	T. & R. ═══ Androstanedione	150° ═══	>700	8				
17-Ethyl(cis)androstanediol-3,17 or 17-ethyl-3-epihydroxy-aetio-*allo*-cholanol-17 	T. & R. ═══ Androsterone	143–144°	38	3				

TABLE 2—*Continued*

NAMES FORMULAE	AUTHORITY* Found in or prepared from	M.P. [α]	PHYSIOLOGICAL ACTIVITY					
			Capon test		Order of potency on rats			
					On minimum weight basis		On capon unit basis	
			γ Equiv. to 100 γ androsterone	Order of potency	Prostate	S. V.	Prostate	S. V.
17-Ethyl(trans)androstanediol-3,17 or 17-ethyl-3-hydroxyaetio-*allo*cholanol-17 	T. & R. ═══ Transandrosterone	204–205° ═══	600	6				
17-Ethylandrostanol-17-one-3 or 17-ethyl-3-keto-aetio-*allo*cholanol-17 	═══ Transandrosterone	137–138° ═══						
Δ⁵-17-Ethylandrostenediol-3,17 or Δ⁵-17-ethylaetio-cholenediol-3-trans-17 	═══ Transdehydroandrosterone	199–200° ═══						

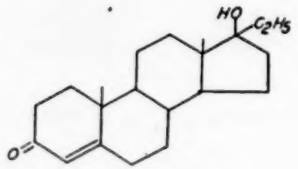
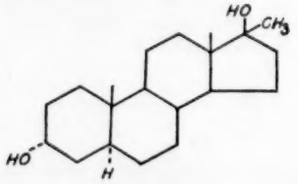
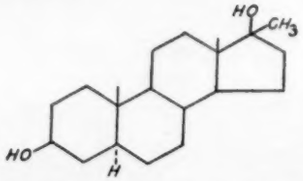
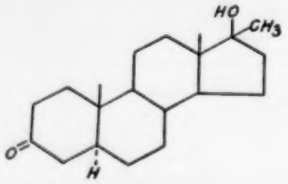
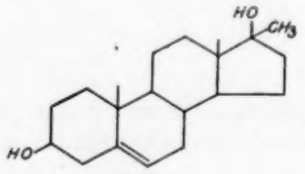
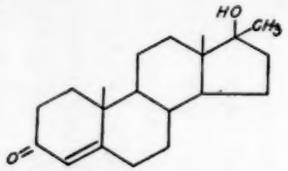
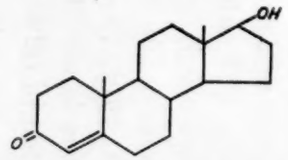
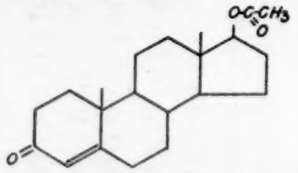
17-Ethyltestosterone or Δ^4-17-ethylandrostenol-17-one-3 or Δ^4-17-ethyl-3-keto-aetiocholenol-17 	17-ethylandrostene-diol	143°						
17-Methyl(cis)androstanediol-3,17 or 17-methyl-3-epihydroxy-aetio-*allo*-cholanol-17 	T. & R. D. & P. Androsterone	184–185° 	38 27	3 2	2 2	3 3	5 5	4 6
17-Methyl(trans)androstanediol-3,17 or 17-methyl-3-hydroxy-aetio-*allo*-cholanol-17 	T. & R. Transandrosterone	211–212°	500	5				

TABLE 2—*Concluded*

NAMES FORMULAE	AUTHORITY[a] Found in or prepared from	M.P. [α]	PHYSIOLOGICAL ACTIVITY: Capon test γ Equiv. to 100 γ androsterone	Capon test Order of potency	Order of potency on rats: On minimum weight basis Prostate	On minimum weight basis S. V.	On capon unit basis Prostate	On capon unit basis S. V.
17-Methylandrostanol-17-one-3	T. & R.	192–193°	25	2	1	2	3	4
or 17-methyl-3-keto-aetio-*allo*cholanol-17	D. & P.		24	2	1	1	6	7
or 17-methyldihydrotestosterone	=	=						
	Androsterone							
Δ⁵-17-Methyl(trans)androstenediol-3,17	T. & R.	204°	525	5	6	6	1	5
or Δ⁵-17-methylaetiocholenediol-3-trans-17	D. & P.		155	5	5	4	1	3
	= Transdehydroandrosterone	=						

17-Methyltestosterone or Δ^4-17-methylandrostenol-17-one-3 or Δ^4-17-methyl-3-keto-aetiocholenol-17 	T. & R. D. & P. ═══ Transdehydroandrosterone	163–164° ═══	25 70	2 3	1 1	1 1	— 2	— 2
Testosterone or Δ^4-androstenol-17-one-3 or Δ^4-3-keto-aetiocholenol-17 	B. & T. T. & R. D. & P. ═══ Testis tissue, cholesterol, androstenediol	154.5–155° ═══ +104–109° at 18°	15 13 16	1 1 1	— 1 3	— 1 2	— 4 9	— 3 8
Testosterone acetate 	T. & R. ═══	139–141° ═══ +87.8° at 20°	13	1	1	1		
Testosterone benzoate		194–196°						

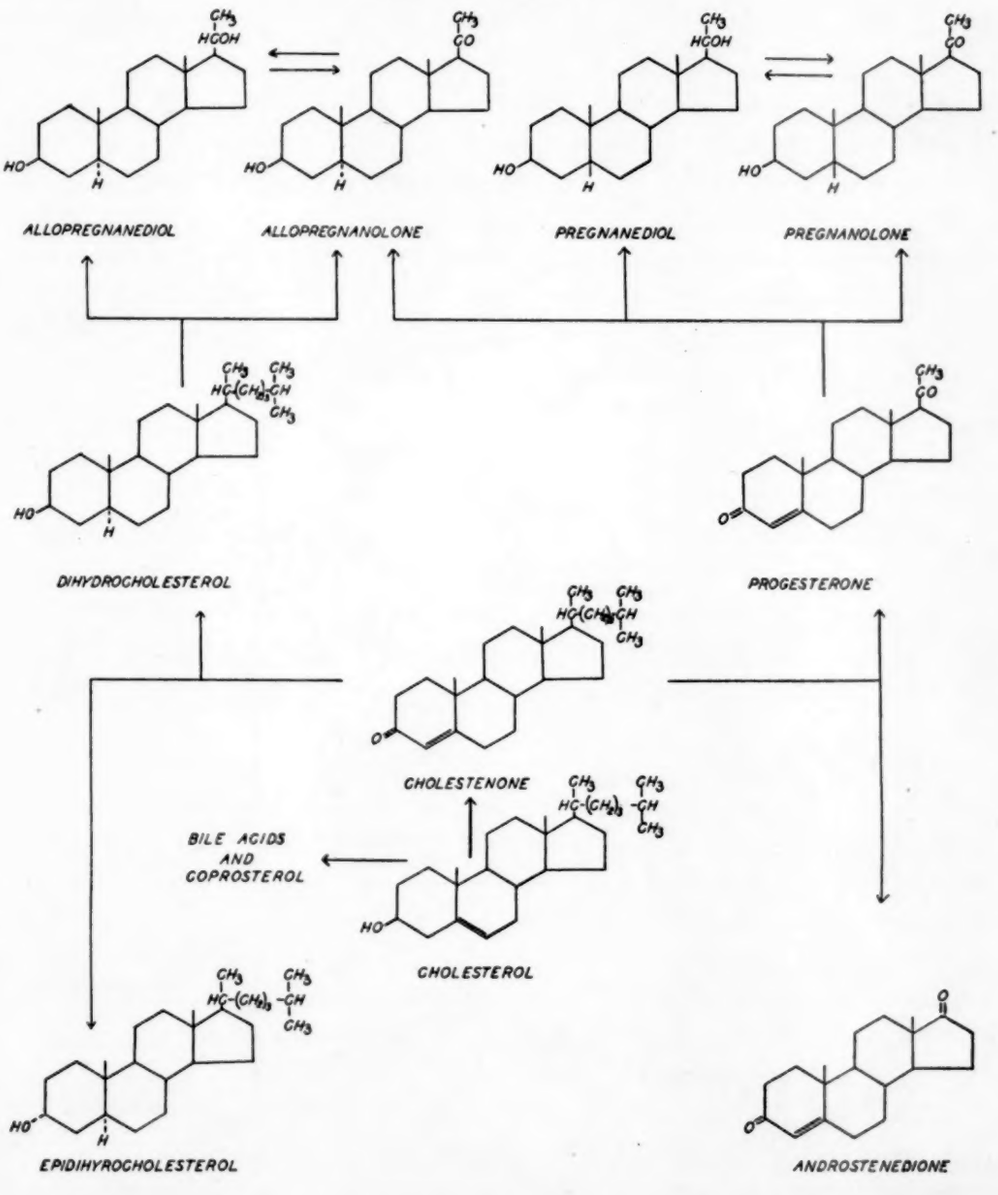
substances of male-hormone character in parallel with the comparative biological activities on the capon and the castrated rat.

The order of activity on the capon has been studied by Butenandt and Tscherning (1935, 1936), Tschopp and Ruzicka (1935, 1936), and Deanesly and Parkes (1935, 1936). Deanesly and Parkes measured the increase in length and height of the comb for their method of assay. The other values are based on the planimetric method. The three groups agree remarkably well on the order of activity and on the dose equivalent to 100γ of androsterone. The only serious exceptions on the dosage basis are Δ^5-androstenediol-3-trans-17-cis and Δ^5-17-methyl-(trans)-androstenediol-3,17, where Deanesly and Parkes obtain about one-third to one-half the values of the other groups, and for 17-methyl-testosterone, where the reverse is true. These discrepancies suggest a qualitative as well as a quantitative difference in the comb reaction, but it is also possible that differences in the time period and in the distribution of the total dose in the various assays as indicated in table 1, may account for the discrepancies. In addition to the substances listed we should consider several other isomers and related compounds. It may be recalled that the two 3-hydroxy-17-keto isomers of androsterone obtained from coprosterol and epi-coprosterol respectively were inactive in the capon in 1-mgm. doses. Tscherning (1936) also reports that Δ^5-3,17-hydroxy-aetio-cholenone-7 as acetate in 2-mgm. doses and Δ^4-17-hydroxy-aetio-cholenedione-3,6 as acetate in 1-mgm. doses are inactive in the capon.

The tabulation of the comparative activities on the basis of mammalian assays seems less justified because the methods are less reliable from a quantitative point of view. The relationship of dose to response in per cent increase in weight of seminal vesicles and prostate in the castrated rat is considered to be expressed by a straight line by Korenchevsky and Dennison (1935) over a dosage range of 200γ to 900γ for androsterone, but for a range of 25γ to 350γ of androstanediol, the curve is hyperbolic. Obviously it is difficult to compare the potencies of different substances when such complications are involved. More extensive data on the mammalian test on a great variety of pure substances as published by Callow, Deanesly, and Parkes (1935, 1936) and Tschopp (1936) reveal this same difficulty. Hence absolute weights of hormones are not indicated in table 2. The order of activities given in table 2 is based on the weights of seminal vesicles and prostates obtained at one to three dosage levels. Different levels had to be chosen because of the necessary widespread in the dosage. In view of these complica-

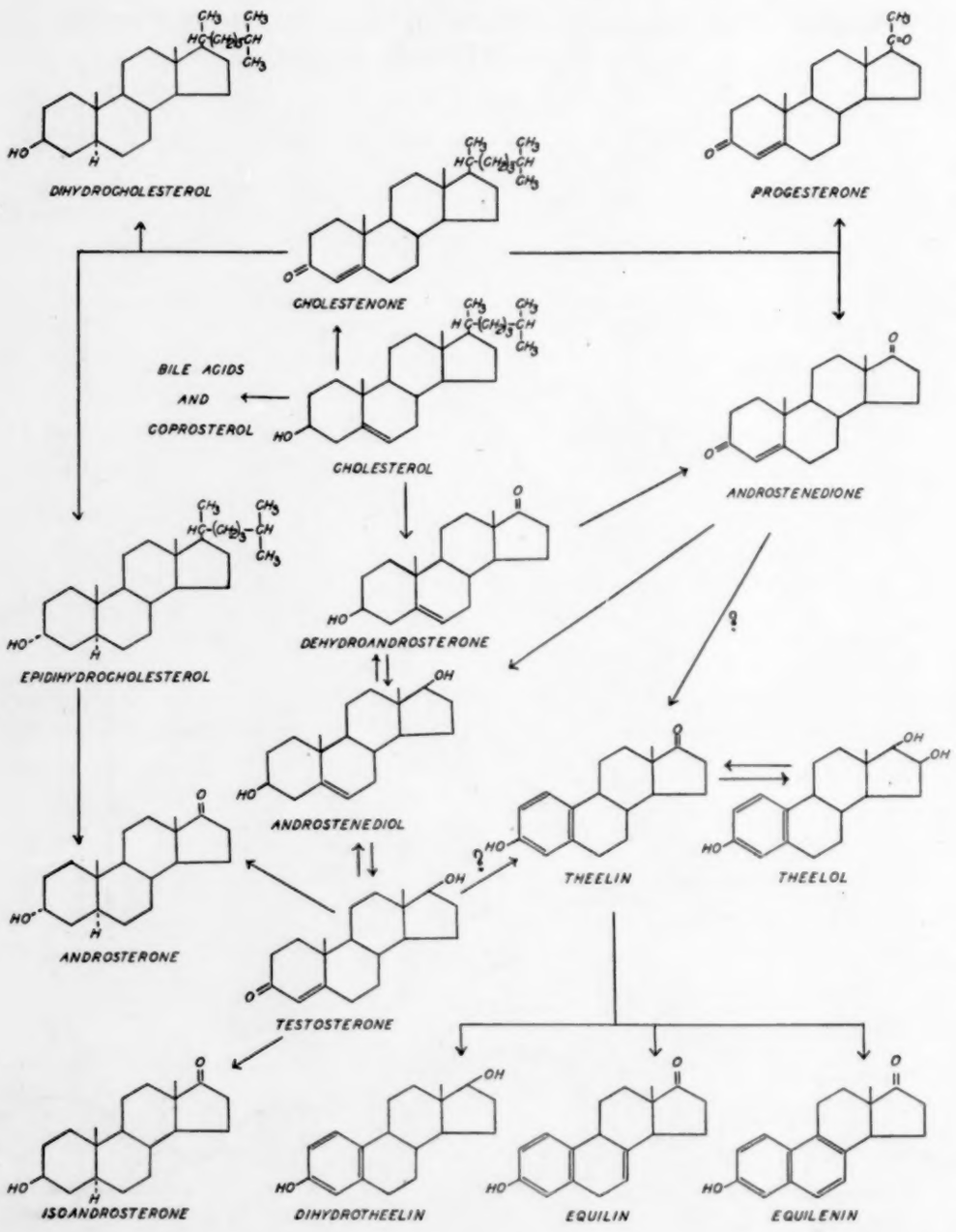
tions the order of activity in the rat based on the capon-unit basis is also of doubtful value.

POSSIBLE BIOLOGICAL ORIGIN OF SEX HORMONES FROM CHOLESTEROL. I



An inspection of table 2 shows that the orders of activity are not the same on capons and rats. In fact they are not even the same for the seminal vesicle and prostate reactions. The former findings are in harmony with the comparative assay findings which the Laqueur group

POSSIBLE BIOLOGICAL ORIGIN OF SEX HORMONES FROM CHOLESTEROL. II



(1931, 1935) reported on various urine and testis-tissue concentrates. A difference in seminal-vesicle and prostate reaction was first observed by Moore, Price, Hughes, and Gallagher (1930) who found the prostate

reaction much more sensitive to testis-tissue concentrates than the seminal-vesicle test in the castrated rat. This was confirmed more quantitatively on the histological basis by Hansen (1933). Recently Callow and Deanesly (1935) compared androsterone, androstanediol, testis-tissue concentrates and urine concentrates. They show that androsterone affects the prostate mainly and the seminal vesicles relatively slightly, but that androstanediol or testis-tissue extract acts about equally well on both accessory sex organs. In their hands, the injection of theelin with androsterone did not correct the effect on the seminal vesicles nor did such combinations bring about, per capon unit of androsterone, the same quantitative effect on the seminal vesicles and prostate as urine concentrates. They, therefore, suggest that other factors are present in urinary concentrates. However, Dingemanse, Freud, and Laqueur (1935) observed that per capon unit the seminal-vesicle reaction from testis-tissue concentrates is greater than from urine preparations. Deanesly and Parkes (1936) report that the relative potency of testosterone over androsterone is five or six times on the capon, two and one-half to five times on the rat prostate, and ten times on the rat seminal vesicle. Tschopp (1936) also calls attention to the greater effect of androsterone on the prostate and states that the normal weight-ratio of seminal vesicle to prostate is in general maintained better by the unsaturated series of male sex-hormones than by the saturated group. Korenchevsky and Dennison (1935) state that in the normal adult rat the weights of seminal vesicles and prostate are approximately the same. In the 30-day castrate rat the average weights for seminal vesicles and prostate are 10 to 12 mgm. and 50 to 55 mgm. respectively. Obviously the rate of regeneration of the two organs when expressed in increase in absolute weight or in percentage increase over castrate weight must be higher in the seminal vesicle than in the prostate if both are to be brought back to the normal weights. Korenchevsky and Dennison (1935) attempted to show the difference in the value of androsterone and androstanediol by expressing the ratio of percentage increase in weight of prostate to that of seminal vesicle in castrate rat on various doses of the hormones. They report that on doses of 200γ to 900γ daily of androsterone, this ratio is 1.06 while for androstanediol on doses of 25γ to 350γ it is 0.64. Obviously they find androstanediol more effective on the seminal vesicles in relation to the effect on the prostate. In fact doses of 25γ androstanediol caused increases in weight of seminal vesicles and prostate of 80 per cent and 48 per cent respectively.

RELATION OF STRUCTURE TO ACTIVITY. Naturally the usual attempts have been made to correlate physiological potency with structure. The correlation is rather poor (Parkes, 1935; Deanesly and Parkes, 1936; and Ruzicka, Goldberg and Rosenberg, 1935).

On capons the negative results with the coprosterol and epicoprosterol derivatives show that in the saturated compounds a 5-cis configuration lowers the activity whereas the 5-trans form or unsaturation increases it. The 3-epi-(cis)-hydroxyl group or a 3-keto group in the saturated series increases the potency considerably over the 3-trans-hydroxyl. This is not the case in the unsaturated series. In general a secondary alcohol in position 17 leads to a more active compound than a tertiary alcohol, but both of these forms are more potent than the 17-keto form.

On rats the 3-epi-hydroxyl form is also more potent than the trans form; however, a 3-keto form is more potent than the 3-epi alcohol derivative. Another difference is that a tertiary alsohol in position 17 is more favorable than either a secondary alcohol or a 17-keto form. Unsaturation in the 4–5 position increases the activity over the corresponding saturated compounds studied thus far.

The potency of esters of various organic acids with various derivatives of the androsterone and testosterone series is of some interest. Butenandt *et al.* (1934, 1935) reported a delayed response in capons from androsterone acetate, androsterone oxime, and androstanediol acetate. Callow and Deanesly (1935) found androsterone benzoate practically inactive in capons and rats. Ruzicka, Goldberg, and Meyer (1935) and Callow (1936) found adrosterone benzoate when given in doses equivalent to 1000γ to 5000γ of androsterone to give irregular and delayed response in the capon. Callow calls attention to the differences in the quantitative but delayed response on such high doses. Deanesly and Parkes (1936) also report testosterone benzoate inactive in capons.[1] Tschopp, however, finds androstanediol monoacetate and diacetate, androsterone acetate, androsterone monosuccinate, testosterone acetate, and androstenediol diacetate all about equal in activity to the respective free hormone forms. He finds one exception in androstanediol disuccinate, which in the capon is only about one-sixteenth as active as the free diol; in the rat the difference does not appear to be so great.

THE ACTION OF ESTROGENIC SUBSTANCES ON THE MALE SEX ORGANS AND ITS SIGNIFICANCE IN THE MAMMALIAN ASSAY AND IN NORMAL PHYSIOLOGICAL DEVELOPMENT. The injurious effects on the testicle of crude

[1] This has been confirmed in the author's laboratories.

lipoid-soluble female-hormone preparations from ovaries, placenta, corpus-luteum tissue, testis tissue, and pregnancy urine were observed repeatedly by Herrmann and Stein (1916, 1921), Fellner (1921), Steinach and Kun (1926), and Laqueur and de Jongh (1928). A careful analysis of the mechanism involved in these studies and independent studies with more carefully purified and standardized hormone preparations led Moore and Price (1930, 1932) to interpret the injurious effect on the testicle of the adult rat as due to the depressing action of theelin or of the male hormone on the gonad-stimulating activity of the anterior pituitary and not a direct sex antagonism action as interpreted by Steinach and Kun (1926). Moore and Price found that in the normal male the theelin treatment leads to degeneration of seminal vesicles and prostate approaching the castrate type with possibly a tendency toward a delay in this process in the castrated male. They conclude, however, that theelin neither stimulates nor directly depresses the accessory male sex organs. Moore and Price did not report a specific type of reaction on the seminal vesicles or prostate of either the normal or castrate rat; furthermore, the combined injection of theelin and male hormone in the castrate produced the same effect on these accessory sex organs as male hormone alone. Their injections were, however, limited to adult rats and to short periods of injection with relatively low concentrations of estrogenic material. More specific effects from estrogenic materials are, however, detectable by using *young* castrated mice or rats or by more prolonged and more intensive theelin treatment of normal or castrated mice or rats of various ages. The more recent studies clearly show this.

Freud (1933) showed that 40 mouse units of theelin or theelol often produced smooth muscle development in the seminal vesicles of young castrated rats especially if injected together with low doses of male-hormone preparations. Lacassagne (1933) injected adult castrated and adult and young male mice with six gamma of crystalline theelin (Girard) over long periods of time. After five months they observed retention of urine and atrophied seminal vesicles, but hypertrophy of the posterior prostate. Independently Burrows and Kennaway (1934) confirmed these observations on normal mice. They applied 0.01 to 0.1 per cent solutions of crystalline theelin (Girard) in benzene by means of a brush to the skin in the interscapular region twice weekly and observed scrotal hernia, atrophy of testicle and seminal vesicles, but enlargement of the posterior lobe of the prostate and obstruction of flow of urine. The stimulating effect of theelin on the seminal vesicles is also

obtained in the hypophysectomized castrated mouse (de Jongh, 1934). David, Freud, and de Jongh (1934) injected immature castrated rats for five to seven days twice daily with 0.2 to 10 gamma of theelin. They found the seminal vesicles, preputial glands, anterior prostate, and periurethral tissue to increase when the higher doses were used, but the histology showed stratified epithelium in place of the spongy columnar type normally found at the orifice of the ducts of the prostate and seminal vesicles. Heringa and de Jongh (1934) also produced a change in the connective tissue layer of the ampulla of the vas deferens by theelin. This consisted in changing the normal woven mass of collagen bundles and network of reticulin in the young castrated mouse to a thicker mucoid type. David, Freud, and de Jongh (1934) as a result of comparative studies with eleven pure estrogenic substances conclude that the estrogenic substances all cause thickening and cornification of the vaginal stratified epithelium in the female and likewise a change from the columnar epithelium to the stratified type in the seminal vesicles and prostate of the castrate. They furthermore find that the corpus-luteum hormone counteracts these effects in the female, but not in the male; however, male hormone counteracts these effects in the male, but not in the female. They consider estrogenic substances to possess three pharmacological actions on the secondary sex organs of both sexes, viz., *a*, stimulation of growth of smooth-muscle tissue and interstitium; *b*, stimulation of submucosa and stratified epithelium growth; *c*, stimulation of growth of submucosa and undifferentiated cylindrical epithelium in non-tubular organs. Korenchevsky and Dennison (1935) studied the effect of 60 to 80 international units of theelin in normal and castrated rats with and without treatment with male-hormone concentrates. Although they found that theelin alone stimulates growth of the seminal vesicles and prostate and causes hypertrophy of the pituitary in the castrated rat, its effect in conjunction with the injection of male-hormone concentrates is primarily on the seminal vesicles. They, therefore, conclude that the rat-prostate method based on weight increase should be considered the equivalent to the comb-growth assay, but that the weight increase for seminal vesicles and prostate together constitutes "the whole male sexual activity" assay. These investigators and Overholser and Nelson (1935) conclude that theelin and the male sex-hormones act synergistically on the epithelial and smooth-muscle elements of the seminal vesicles. Callow and Deanesly (1935) and Tschopp (1936) do not consider the theelin action as a pacemaker or synergist. The data, however, indicate that

theelin acting with testosterone or adrosterone in the young castrate rat produces greater increases in the weights of seminal vesicle, prostate, Cowper's and preputial glands than the sums of the independent actions of theelin and testosterone, thus in part supporting Korenchevsky's statement.

The fact that these estrogenic effects are obtained more easily in the young castrates on relatively low doses or in normal adult rats and mice after more prolonged treatment suggests that male sex-hormones may counteract these actions somewhat. Studies by Pfeiffer (1936) show that a slight development of smooth muscle takes place in the seminal vesicles of the male rat if castrated at birth, but that such animals are more susceptible to a smooth-muscle reaction in the seminal vesicle as a result of ovarian grafts. If the animals are considerably older or adult before castration, the ovarian transplants do not bring about the smooth-muscle reaction so readily. Pfeiffer suggests that the differentiation of the seminal vesicles in the more mature rats renders them more refractory to the theelin action. Wells (1936) also observes an inhibiting action of male hormone to theelin in the ground squirrel. During the season of low sexual activity, he finds theelin to produce marked hypertrophy in the accessory sex organs of normal as well as of castrated males. The character of the hypertrophy is of the nature previously described. During the breeding season these effects are less marked in the normal male but the accessory sex organs are injured to some extent. The interpretation is that during the season of sexual activity the male-hormone secretion depresses the theelin action. More direct evidence is given by Zuckermann and Parkes (1935, 1936) who found theelin to produce fibromuscular growth of the prostatic stroma and epithelial stratification and distention of the uterus masculinus in the immature monkey. When androstanediol (50 parts) was injected with theelin (1 part) the theelin effects on the prostate were suppressed. Progesterone (20 parts) did not suppress the effect from 1 part of theelin. van Wagenen (1935) also observed the smooth muscle hypertrophy of the seminal vesicles in immature monkeys as a result of 34 days' treatment with theelin. de Jongh (1935) found three capon units of testis-tissue concentrate daily to counteract the prostatic reaction from three gamma of theelin in the rat. Laqueur (1934) has attempted to apply these findings to prostatic hypertrophy in man on the assumption that in these patients there is an improper ratio or balance between male and female sex-hormones resulting in a relatively higher concentration of the estrogenic material than of androgenic activity. He reports

favorable results in about 60 per cent of the cases of prostatic hypertrophy treated by male-hormone therapy. Hamilton, Deming, and Allen (1936), however, found no estrogenic material in the urines of twelve patients before and after prostatectomy. Inasmuch as the urine of normal men always contains estrogenic material in appreciable quantities, these negative findings are very surprising.

It is obvious from the above considerations that we cannot be too cautious in the interpretation of biological assays made with crude extracts without the actual removal of the oestrogenic (phenolic) fraction from the androgens or non-phenolic fraction.

OTHER PHYSIOLOGICAL STUDIES WITH PURE AND IMPURE MALE SEX-HORMONES. I. *Action on other accessory sex organs.* a. *Cowper's gland.* Tschopp (1936) also found the increase in weight of Cowper's glands in the castrate rat to respond in about the same order as the increase in weight of the seminal vesicles after injection of various pure androgens. The absolute increase in weight is greater, but the percentage change less than for seminal vesicles. Testosterone was most potent and transdehydroandrosterone least.

b. *Penis.* The increase in weight of the penis in the castrate rat has been studied by Korenchevsky, Dennison, and Kohn-Speyer (1932, 1933, 1935), by Tschopp (1936), and by Ito and Kon (1935). Korenchevsky and Dennison find the average per-cent increase in weight of the rat penis per 100γ androsterone for doses of 200 to 900γ to be 11 per cent and per 100γ androstanediol for a range of 25 to 200γ to be 100 per cent. Tschopp reports similar results and essentially the same order of activities for the various androgens as found on the increase in seminal-vesicle weight. Ito and Kon (1935) observed the effects from the high doses of a urinary concentrate in normal puppies and young rats after three to six weeks' and two months' treatment respectively. In the puppies, they observed marked increases in the weights of all the accessories, a fall in testis weight and marked erections. After six weeks, a fall in body weight was recorded. In young rats, they produced a protrusion of the enlarged penis, a partial cornification of the mucous membrane and the accumulation of coagulated semen therein. With loss in body weight and inhibition of thymus growth, they also observed hypertrophy of the liver.

c. *Preputial tissue.* The preputial tissue response has been studied by Voss (1931), Freud (1933), Korenchevsky et al. (1934, 1935), and Tschopp (1936). In castrated mice the testis-tissue concentrates caused regeneration of, and produced mitosis in the preputial glands

(Voss). In castrated rats, an increase in weight of the tissue was observed from 2 capon units daily and no effect from 30 mouse units of crystalline theelin (Freud). High doses of crystalline theelin (100γ or more) caused growth of the tissue and low doses enhanced the effect produced by 2 capon units of male-hormone concentrate. Korenchevsky and Dennison, however, report increases of 13 per cent and 39 per cent from 60 to 180 units of theelin in the castrated rat. With androsterone in doses of 200 to 900γ and androstanediol in doses of 25 to 200γ, they observed average 18 per cent increases in weight of the tissue per 100γ of androsterone and 37 per cent per 100γ of androstanediol.

d. *Periurethral tissue.* Growth of periurethral tissue is also stimulated by androgens. Freud and Laqueur (1934) report this action in the immature male rat from urine concentrates on doses which had little or no effect on the seminal vesicles. Korenchevsky (1935, 1936) finds that 175 to 700γ of adrostanediol daily in the spayed rat caused development of otherwise inconspicuous periurethral tissue which is similar in structure to the prostate. Theelin did not produce this development.

e. *Estrogenic action in the rat.* 1. *Theelol-like action.* Butenandt and Kudszus (1935) and Butenandt (1936) suggest that the unsaturated series of androgens show more theelol-like action than the saturated series. They find 400 to 2000γ daily for 3 days, of androstenedione, dehydroandrosterone, or testosterone to produce vaginal introitus and estrus in the normal infantile rat. In doses of 70γ per day for 3 days, Δ^5-androstenediol produced the same effect. Androsterone in 2000γ doses was found inactive. Tschopp (1936) and Deanesly and Parkes (1936) confirmed these conclusions on adrostenediol and testosterone. The latter authors injected once daily for 2 days and find the total doses of the following to give the theelol-like action: androstanediol (2 to 4 mgm.), dehydroandrosterone (2 mgm.), transandrostenedione (2 mgm.), methyl transandrostenediol (0.5 mgm.), methyl androstanediol (0.5 mgm.), 17-methylandrostanolone-17,3 (0.6 mgm.), and methyl testosterone (3 mgm.). They also report negative results with androsterone (2 mgm.). Obviously even the saturated androgens also possess a theelol-like action on the normal immature rat. It appears that androgens in high doses, as compared with theelin or theelol, do act upon the female sexual organs.

2. *Theelin-like action on the spayed mouse or rat.* Butenandt (1936) found four injections of 200γ each of Δ^5-androstenediol in a period of 36 hours in spayed mice to produce estrus which lasted for 1 to 2 days. Deanesly and Parkes (1936) report similar high doses of androstanediol,

dehydroandrosterone, androstenediol, methyl transandrostenediol, and methyl androstanediol to produce estrus in the spayed immature rat. The following have given negative results thus far: androsterone, androstenedione, testosterone, and methyl testosterone. Warren (1935) independently confirmed the nonestrogenic activity of androsterone (1000γ) in spayed mice. Tschopp (1936) independently also found transandrostenediol and its 17-methyl derivative to produce estrus in the spayed adult rat. Tschopp (1936) also observed growth of the uterus after such treatment. Korenchevsky, Dennison, and Simpson (1935) found 22 to 27 days' treatment of spayed adult rats with androsterone or androstanediol to cause increase in weight of the uterus and vagina. The effect from androsterone (1.8 to 3.6 mgm. daily) was slight on the uterus and negative on the vagina; from androstanediol (0.49 to 0.62 mgm. daily) very significant increases in weights were obtained, but the recovery was not complete. Korenchevsky (1936) likewise finds testosterone to stimulate growth "of all the atrophied sexual organs" in the ovariectomized rat.

f. *Progesterone-like action.* The close structural relations existing between progesterone and some of the unsaturated Δ^4-androgens naturally suggests possible similarity in physiological action. These studies have been very limited, mainly because the doses even of progesterone are high. However, Klein and Parkes (1936) find the normal immature rabbit to give responses equivalent to 0.25 to 0.5 mgm. progesterone on 10 to 20 mgm. of methyl androstanediol, methyl-trans-androstenediol, methyl dihydrotestosterone, and methyl testosterone. Testosterone in 20 mgm. doses was less potent. It must be emphasized that the doses used are 40 to 80 times those of progesterone and that the rabbits were not ovariectomized. All other studies (Butenandt et al., 1934–36) indicate that progesterone is very specific if dosage is properly controlled.

g. *Mammary glands.* Selye, McEuen, and Collip (1936) report that 200γ testosterone benzoate daily for 14 to 23 days caused mammary gland growth in normal and gonadectomized male and female rats.

II. *Effects on other organs.* a. *Thymus.* The inhibiting action of thymus implants or extracts on the estrous cycle in the normal mouse were demonstrated independently by Walter (1930) and Loewe and Voss (1931) and clearly indicate an antagonism between the ovaries and thymus. The studies of Evans and Simpson (1934) involving the chronic treatment of normal and castrated rats with gonadotropic hormone also show that the ovaries and testes are involved in inhibiting the growth of the thymus in the immature normal animal and in hasten-

ing the atrophic changes in the normal adult. Korenchevsky, Dennison, and Simpson (1935) showed that androsterone or androstanediol hasten the involution of the thymus in the castrated male and spayed female rats. Testosterone was found to show the same action. With impure testis-tissue extracts or theelin the results were more irregular. Ito and Kon (1935) observed the same effects on normal immature rats from a urinary male-hormone concentrate.

b. *Suprarenals.* Further evidence on interrelationships between the adrenals and gonads is accumulating. Cannon (1932) claims that adrenal cortex contains a substance which inhibits the gonads of mature albino rats, but Corey and Britton (1931) report that adrenal-cortex extracts induce precocious maturity in the same species. Eaton, Insko, Thompson, and Chidester (1929) find both activities in the suprarenal gland because by feeding dried suprarenal cortex to chicks they developed larger testes, but dried suprarenal-medulla-fed chicks developed smaller testes than normal. Oskar Klein (1931) also finds that adrenalin-free cortical extracts stimulate development of the gonads in the young male but inhibit sexual development in the young female. Handovsky and Tammann (1928) also find a sex difference in the changes produced in carbohydrate metabolism in rabbits when adrenalectomy follows castration. Schiller (1935) reports that sex function was interrupted in six male and fifteen female rats after three days following complete adrenalectomy. Daily adrenalin injections are said to have brought about a return to the normal condition.

Korenchevsky, Dennison, and Simpson (1935) could maintain the normal suprarenal weights in gonadectomized male and female rats by 1.8 to 3.6 mgm. of adrosterone and 0.17 to 0.7 mgm. adrostanediol respectively. Theelin in daily doses of 180 international units was less effective in inhibiting the adrenal hypertrophy. In fact, in normal male rats, 43 days' treatment with daily doses of 60 to 180 international units of theelin caused hypertrophy of the suprarenals.

That the suprarenals may produce an androgenic substance is indicated by two lines of research. The first is that women with adrenal tumors present marked hirsutism and excrete abnormal amounts of androgenic material. The second is that three independent workers (Kendall, Wintersteiner, and Reichstein, 1936) have prepared an oxidation product from an adrenal-cortical principle which is found to be one-sixth to one-fourth as active as androsterone in the capon test.

c. *Liver.* In 1934 Korenchevsky and Dennison reported that in the castrated male rat, the liver decreases from the normal average weight

of 11 grams to 9.5 grams. A year later with Simpson they restored these weights almost to the normal weight by the same doses of adrosterone and adrostanediol used for the adrenal control referred to above. The same effects were produced in ovariectomized rats. No definite histological changes were reported.

d. *Kidneys and heart.* The same investigators report that gonadectomy leads to a slight fall in the weights of kidneys and heart. In both sexes, the normal average weights are almost regained by the prolonged use of androsterone or androstanedial as indicated under suprarenals above.

e. *Sebright feather-pattern control.* It is well known that the hen-feathered Sebright bantam possesses the same plumage in both sexes, but that castration leads to the capon type. Gallagher, Domm, and Koch (1933) injected highly purified male-hormone concentrates into Sebright capons and produced typical hen feathers. This was true even when the concentrates were freed from estrogenic material. Callow and Parkes (1936), however, could not produce hen-feathering in their Sebright capons by 0.5 to 1 mgm. of androsterone, whereas 1 to 2 gamma theelin brought about the change. They, therefore, conclude that the normal plumage of the Sebright cockerel is controlled by the secretion of estrogenic material. Gallagher, Domm, and Koch (1936), however, conclude that the estrogenic material is not necessary because they produced hen-feathering with androsterone, obtained by the synthetic degradation of cholesterol.

III. *The X substance of the Laqueur school.* This substance, found in testis-tissue extracts, when injected alone, shows no action on the seminal vesicles and prostate in the castrated rat, but when administered with a given dosage of testosterone it acts as an activator. This activation is not the same as the synergistic effect of theelin in its action with androsterone on the seminal vesicle because the X substance is inactive alone and because it does not activate androsterone. The substance also appears to activate dihydroandrosterone in the mammalian test. In hypophysectomized young castrated rats, the X substance also has no effect on the androsterone action, but the activating actions toward testosterone and dihydroandrosterone are the same as in the castrate. The X substance is non-estrogenic in character. The activity has been found in testes, liver, suprarenals, ovaries, blood (cow and gull), wheat seedlings, and urine. The lowest dosage found active thus far on the purest known form is 1 milligram. It is lipoid soluble. The main

observations on this factor were first announced by David, Dingemanse, Freud, and Laqueur (1935), Freud (1935), Freud, Dingemanse, and and Polak (1935), and were later confirmed by Ruzicka, Wettstein, and Kägi (1935), Tschopp (1936), Callow and Deanesly (1935), and Deanesly and Parkes (1936). These observations probably also explain in part the discrepancies many of us have observed between capon and mammalian assays in various fractionation studies on urinary and testis-tissue concentrates.

IV. *The testis-hormone control of the anterior pituitary.* Innumerable clinical and experimental studies (for the earlier literature, see Biedl, 1913, and Lehmann, 1927) have established that gonadectomy in both sexes leads to hypertrophy of the hypophysis which is associated with characteristic histological changes in the anterior lobe and with alteration of the physiological activity thereof. The histological studies reveal an increase in the number and size of the basophiles which later undergo vacuolization with development of "signet-ring" or "castration" cells. The changes in physiological activity involve increased content or secretion of gonadotropic hormones as demonstrated *a*, by implantation of the hypophysis into normal immature animals; *b*, by the higher concentration of gonadotropic substances in the urine of certain species after castration, and *c*, by parabiosis studies on gonadectomized rats and mice united with normal animals of either sex and observing the changes in the gonads of the normal mate and in the accessory sex organs in both animals.

Is the anterior pituitary lobe under the control of the male sex-hormones which have been separated thus far or are other substances from the gonads more effective alone or together with the androgens now known? These questions cannot be answered satisfactorily at the present time. The discussion below very definitely indicates that several factors may be involved in the testis control of the anterior pituitary activity. The solution of these problems is complicated by the fact that the quantiative biological studies on the hypophysis yield exceedingly variable results on the basis of weight, histological and physiological changes in the pituitary and that these fluctuations are affected by age, sex, season, and species. An extensive survey of the literature on all phases of this problem cannot be undertaken here. Instead, the review is limited to a consideration of the effects of various extracts, concentrates, and pure hormones on the anterior pituitary. The effects will be considered on the basis of *a*, histological changes; *b*,

sex antagonism studies; *c*, changes in physiological activity of the anterior lobe as determined by implantation studies, and *d*, changes in physiological activity as determined by parabiosis studies.

a. *Effects of extracts on the histology of the anterior pituitary lobe in the castrate.* Fichera (1905) made the most remarkable claims as to the effect of extracts from cockerel testes on the pituitary in the capon 20 to 25 days after castration. He stated that his extracts produced marked restitution to the normal histology as early as 10 to 15 hours after the first or second injection. Fichera no doubt employed very crude and dilute extracts and very likely his histological methods were equally non-critical.

Lehmann (1927, 1928) from histological evidence on serial sections of the anterior pituitary in gonadectomized rats concluded that male castrates' pituitaries are corrected by implants or extracts of testis tissue, but not by transplants or extracts of ovaries. He claimed the same sex specificity for ovarian transplants and extracts in the spayed female rat. His extracts represented the crude non-saponifiable fraction, but nothing is stated as to the dosage employed. He concluded that the anterior pituitary possesses a marked sex specificity. In his second paper he found no sex differences in the treatment with placental extract injections or placental tissue by mouth and hence concluded that placental tissue contains male and female sex-hormones.

The qualitative sex specific reaction of the anterior pituitary body to sex hormones suggested by Lehmann (1927) have not been confirmed throughout. However, it is very evident that a sex difference of a quantitative character does exist in some species. This will be stressed from time to time in the later developments discussed below.

Hohlweg and Dohrn (1931, 1932) prevented the histological changes in the anterior pituitary lobe of gonadectomized rats of both sexes by the injection of Progynon. However, it required twelve times as much for the male as for the female and more for adult than for young castrates. The Progynon used may not have been free from male hormone and hence the positive action on the males might be due to the contamination. This would also explain why so high a dosage was necessary for males. More recent studies with pure preparations eliminate the validity of these suggestions.

McCullagh (1932) states that the doses of testis tissue or urinary hormone concentrates (lipin fraction) which correct the castration changes in the accessory sex organs of the castrated rat do not prevent the appearance of "castration cells" in the anterior pituitary, but that

water extracts from much less testis tissue correct the pituitary changes. He also emphasizes that with destruction of the germinal epithelium one observes the appearance of castration cells without the degenerative changes in the accessory sex organs. However, later McCullagh and Walsh (1935) in parabiosis studies find the lipin-soluble male-hormone concentrates to inhibit the gonad-stimulating activity of the castrated animal.

A series of studies by Nelson and collaborators has done much to clear up the quantitative aspects of some of the questions raised above. At one time Nelson (1934) was inclined to believe that only the estrogenic impurities in the lipin-soluble androgen preparations inhibited the anterior pituitary or prevented the appearance of castration cells in the castrated rat, but concluded (1935) that the male pituitary requires 5 to 6 times as much crystalline theelin for inhibition as the female. However, Nelson and Gallagher (1936) also find male-hormone concentrates, free from estrogenic material, to correct or prevent the castration changes in the pituitary, in both sexes. The differences in doses of the same preparation required to produce the same inhibition in the pituitaries of the two sexes are again observed. The relative efficiencies of estrogenic and androgenic substances are also very striking. They find that when the male-hormone concentrate contains traces of estrogenic activity (alkali soluble) the 30-day castrated female rat requires 2 capon units per day for pituitary control but that the male castrate requires 10 capon units under comparable conditions. This is the 1 to 5 ratio found for crystalline theelin. However, when the male-hormone concentrate is thoroughly freed from alkali-soluble estrogenic material, the dose of male-hormone concentrate required for the spayed female rat must be increased to 10 capon units per day to prevent histological changes in the anterior pituitary in 30 days. What dosage of the same estrogen-free material is necessary for the castrated male has not been determined. Nelson and Gallagher (1936) also show that the addition of 0.78 rat unit of crystalline theelin to 4 capon units per day is as efficient in the spayed female as 10 capon units alone and better than either 7.5 capon units or 0.78 estrogenic rat unit alone. Furthermore, the estrogenic-free male-hormone concentrates from urine and testis tissue were equally effective per capon unit in the spayed female. This is especially remarkable when we recall the low order of potency of androsterone, as capon units, as compared with testosterone in restoring the accessory sex organs in the castrated male rat. The relative efficiency of theelin and male-hormone concentrates on the pituitary suggests a

biological rôle for the presence of estrogenic material in the male. In fact, Nelson and Gallagher (1935) consider it so effective that it is not necessary to hypothecate another male hormone for pituitary control. In other words, the combined action of the known androgenic and estrogenic substances is sufficient to account for the natural control of the anterior pituitary body.

b. *Sex antagonism studies—Injury to the gonads.* From time to time attempts have been made to demonstrate the feminizing and masculinizing actions of the sex hormones. Naturally the earlier studies involved the effects from gonad transplants. The leaders in these earlier studies (Steinach, 1910–20; Sand, 1922; Moore, 1922, and Lipschütz, 1925) arrived at very different conclusions and interpretations of their findings. Steinach and Lipschütz independently interpreted the results as due to direct sex-hormone antagonism. Sand did not subscribe to this interpretation but in fact claimed that a heterogonad transplant was successful only when implanted into the gonad tissue. Moore could not demonstrate true sex antagonism by transplantation studies because he succeeded in keeping transplants viable in various sites, immaterial whether the opposite gonad was left intact or removed. The loss of spermatozoa production in testis transplants, Moore could account for on the basis of temperature effects. He concluded that the internal secretion of the testis transplant was not inhibited by the intact ovary and that the intact ovary still continued to function normally.

The earlier studies with sex-hormone preparations also tended to support the sex-hormone antagonism hypothesis. Thus, Fellner (1921) showed that the injection of large doses of the crude lipin fraction from placenta, ovaries, or corpus luteum caused damage to the spermatogenic elements, but not to the interstitial tissue in the rabbit testicle after the previous removal of one testis. Fellner also observed that a similar extract from bull testis-tissue (equivalent to 4 to 11 bull testicles!) produced the same effect and stimulated development of the mammary glands. Stein and Herrmann (1921) reported similar effects from corpus luteum and placental extracts on the testes of rats, guinea pigs, and rabbits, but they also called attention to the atrophy or degeneration of the accessory sex organs and stimulation of the mammary glands. The effects were interpreted as evidence of sex-hormone antagonism brought about by the "female sex-hormone" in the various extracts studied. However, a present-day interpretation is that the action is due to the inhibiting action of either male or female sex-hormone on the pituitary as a result of which the normal gonadotropic activity of the

anterior pituitary is not manifested in the intact gonad and thus, secondarily, the accessory sex organs degenerate.

With the production of better standardized sex-hormone preparations of higher purity and potency and especially with the actual purification of the male-hormone concentrates, more quantitative studies on so-called sex antagonism and on pituitary inhibition became possible. The importance of purity cannot be overemphasized. There is considerable evidence that various extracts or lipoid fractions which are entirely free from estrogenic and androgenic actions nevertheless may show so-called sex antagonism action. No doubt this factor is involved in the studies cited above and in Lendle's (1931) attempt to standardize crude male-hormone preparations on the basis of the inhibition of estrus in the normal rat. Studies by Walter (1930), Loewe and Voss (1931), and by Duncan, Gallagher, and Koch (unpublished) all show that estrus may be inhibited in the adult rat by various lipin preparations obtained from various sources, but free from or negligible in content of sex hormones. Moore and Price (1932) also call attention to the injurious effect of lipins from heart, liver, and brain tissue on the rat testis. Whether these sex-hormone-free lipins act directly on the intact gonads or indirectly through the pituitary has not been determined. Obviously injury to the testis morphology or activity is not limited to female sex hormones nor is the mechanism of the action necessarily the same for different substances. In any case the dosage and the time of treatment employed are very important considerations. Although it appears well established that estrogenic and androgenic substances inhibit the gonadotropic activity of the anterior pituitary body of both sexes and thus injure the gonads of both sexes indirectly, it does not necessarily follow that certain end organs may not be modified in type by the direct action of a sex hormone of the opposite sex. This is well illustrated by the experimental hermaphrodism produced by Voss and Loewe (1931) in the female guinea pig by crude male-hormone injection and by the simultaneous stimulation of comb growth and female plumage pigmentation in the Brown Leghorn fowl (Juhn, D'Amour and Womack, 1930) with mixtures of male and female sex hormones.

That the anterior pituitary is involved in the so-called sex antagonism observations is suggested by numerous preliminary studies, but the final correlation of these data and the application thereof to the problem of sex antagonism remained for Moore and Price (1930, 1932). That no direct antagonism between the male and female sex hormones exists was demonstrated by Moore and Price when they found a given dose of

male hormone to control the accessory sex organs of a castrate equally well with or without 12 rat units of estrogenic material. They call attention to similar evidence by Ihrke and D'Amour as to the negative effect of male hormone on the estrogenic action of a given dose of "estrin" in the spayed rat. In the intact male rat they found 12 rat units of "estrin" (from placental tissue) to inhibit the testis as shown by the degeneration of the seminal vesicles. This "estrin" effect was, however, easily counteracted by the injection of urinary gonad-stimulating material or by hypophyseal implants thus showing that the testis had been inhibited due to the lack of gonad-stimulating secretion from the pituitary gland. They also showed that 6 capon units of male hormone caused a similar injury of the immature rat testicle; this is also corrected by hypophyseal implants or by urinary gonadotropic preparations. The above and a great mass of similar evidence from their own experiments and from those of others were correlated and led them to announce the generalization that sex hormones inhibit the heterologous and homologous gonads only indirectly by first inhibiting the gonad-stimulating activity of the anterior pituitary lobe. In other words, so-called sex antagonism really does not exist because either sex hormone may inhibit the anterior pituitary of either sex and hence the gonad of either sex. Moore and Price, however, did not investigate the quantitative differences in the pituitary response of the different sexes to the sex hormones. Schoeller and Gehrke (1933) confirm the effect of crystalline theelin or of urinary male-hormone concentrates on the testicle of the young rat. In the adult rat, neither Moore and Price nor Schoeller and Gehrke observed the decrease in size of the testes after male-hormone treatment. In further support of the Moore and Price interpretation we have the observations by Freud (1932) that in the hypophysectomized rat, the sex hormones produce the normal reactions in the accessory sex organs but do not produce antagonistic action. Walsh, Cuyler, and McCullagh (1933) also found the urinary male-hormone concentrate to prevent the atrophy not only of the seminal vesicles and prostate but also of the testes in the hypophysectomized rat. These observations were based on only one sex-hormone treated animal, one normal control, and one hypophysectomized control.

The age and sex specificity characteristics of the anterior pituitary can be reintroduced to advantage at this point. The action of the sex hormones on the anterior pituitary lobe is not quite as simple as suggested by Moore and Price, if the findings of Sand (1918), Smelser

(1933), Hisaw et al. (1934), Wolfe (1935), Clark (1935) and Pfeiffer (1935, 1936) are significant. Sand (1918) and Smelser (1933) find that ovarian grafts in male guinea pigs never luteinize, but do so in the female. Hisaw et al. (1934) and Wolfe (1935) state that "estrin" injected into females in proper amounts causes the release of the luteinizing factor from the anterior pituitary and the former group concludes that "estrin" suppresses the follicle-stimulating factor only. These studies suggest that the adult male anterior pituitary of the rat contains the follicle-stimulating hormone and little or no luteinizing factor. Hellbaum (1935) demonstrated the same difference for young mare and stallion pituitaries. Clark (1935) has demonstrated the difference in gonad-stimulating power of hypophyseal implants in immature mice from rats of both sexes at different ages and also from the gonadectomized rats at different ages. She finds the anterior pituitaries from normal females to be more potent from birth to 20 days of age; the normal male anterior pituitary gradually gains in potency until the age of puberty when it becomes more active than the normal female ever appears to be. If rats are gonadectomized on the first day of life and their pituitaries then assayed sixteen to eighteen days later, the sex differences in gonad-stimulating activities of the pituitaries are negligible. However, the castrated males possess pituitaries which average an increased activity of 102 per cent over the normal males. In the females, the activity was increased on an average by 19 per cent as a result of ovariectomy. Pfeiffer (1935, 1936) has demonstrated that in the immature rat the anterior pituitary lobe has not differentiated, that potentially it may become either male or female in type and that the type it differentiates into is determined by the type of gonad secretion influencing it. Pfeiffer was able to convert the bipotential anterior pituitary of a prepuberally castrated male or female rat into the female or male type by implanting ovary and testis respectively. Lipschütz and Villagran (1935, 1936), however, report that normally the male hypophysis has 30 times the luteinizing power of that of the female, but that castration leads to a decrease of the luteinizing power in the male and an increase therein in the female. Lipschütz and Del-Pino (1936) find only slight differentiation of the anterior pituitary in man, except in cases of advanced pregnancy. These contradictory conclusions are no doubt due to different experimental procedures and probably also involve very different quantitative relations from the standpoint of the hormones involved. With the pure preparations of sex hormones now

available we can expect to control this variable better and it is to be hoped that the very suggestive conclusions of Pfeiffer may be confirmed more definitely.

The reciprocal relation between certain end-organs and the testicles. There are many observations on the effects of light, temperature, diet, nutrition, etc., on the control of the size and activity of the testicles. Hibernation involves these factors and light is the most important in many species. In the ferret (putorius vulgaris), Bissonnette (1935) considers light to act through the pituitary on the germinal and interstitial cells of the testes, but he considers ferrets as well as other animals with definite seasonal sexual cycles to possess an "inherent sexual rhythm." He showed that the eyes are involved in the light control of the hair cycle in the female ferret. Johnson and Hanawalt (1930) conclude that hibernation in the thirteen-lined ground-squirrel is not altered by the injection of thyroxin or pituitrin or by feeding desiccated thyroid or thymus tissue in large doses. Zalesky (1935), from his studies, concludes that the distinct seasonal changes of the thyroid in this species are not correlated with the sexual cycle because castration or administration of sex hormones from pregnancy urine or other gonad-stimulating preparations had no observable effect on the thyroid. However, the administration of anterior pituitary substance during the season of low thyroid activity stimulated the thyroids to a higher level of activity than the highest in the normal rhythm. Wells (1935) and Wells and Moore (1936) also conclude that a periodic activity of the anterior pituitary is involved in the sexual rhythm in this species, but they found no evidence that light is a factor. However, they found that the testes and accessory sex organs can be stimulated to an active condition during the sexually inactive period. An incidental and interesting observation is that crystalline androsterone produced precocious spermatozoön formation in the immature ground-squirrel and also earlier than normal in the sexually inactive adult. Baker and Johnson (1936) confirmed the positive effects from urinary gonad-stimulating preparations in the sexually inactive ground-squirrel.

In birds and fowl, light or season plays a very important rôle in the size of the gonads and other endocrine organs. Riddle and Fisher (1925) called attention to the marked seasonal variation in the weights of the gonads and thyroids in pigeons and ring doves. No doubt light is an important factor in the gonad control in this species. In the common starling (sturnus vulgaris), Bissonnette and Wadlund (1932) found the control of gonad size spread over a wide range of wave lengths of

visible light and suggested that the anterior pituitary is involved. The most striking observations are reported by Buckner, Insko, and Martin, (1934) who observed that when white leghorn cockerels are raised without exposure to sunlight they develop exceptionally large and less erect combs, but relatively small testicles. However, when the cockerels are raised exposed to sunlight, their combs are smaller and more erect, but the testes are very large. Furthermore, removal of combs and wattles produced cockerels with small testes whereas in pullets no such effect was observed on ovarian weight. The studies by Womack and Koch (1931) and Koch and Gallagher (1934) show that the comb is a more refractory end-organ to androgens when the capon is exposed to light than when kept in dim light. This suggests that the larger comb developed in the normal cockerel in dim light calls for less male sex-hormone and hence the testes are smaller. However, it does not necessarily follow that a smaller organ is secreting its hormone less rapidly. That the comb may act antagonistically on the testes is also shown by M. M. Zawadowsky (1935) who confirmed the observations of Buckner et al. that comb removal results in larger testes in cockerels. Zawadowsky (1935) also observed slight atrophy of the seminal vesicles and prostate in rats after feeding cocks' combs by mouth. Jores (1935) reports that the melanophore hormone-content of the pituitary body and eyes of white mice is 2 to 3 times greater in animals kept in the dark than it is in controls kept in light. He claims that the same relations hold for the content of pressor and oxytocic activities in guinea pigs' pituitary glands with and without light treatment. Hence, he concludes that the light control of the pituitary is through the eye. Further observations confirming the pituitary or light factor or both on the gonads were made on the sparrow by Kirschbaum and Ringoen (1936), and Witschi and Keck (1935), and by Warren and Scott (1936) on ovulation in the fowl.

Other interesting studies involving damage to the testes are presented herewith. Buchheim attempts to explain the loss of sexual desire in the firemen of the French navy as due to a harmful effect of fuel oil because he finds that he can produce the same changes in rats, guinea pigs, and rabbits by injecting 0.005 cc. or feeding 0.115 cc. "mazout." In rats, he observed atrophy of the seminal vesicles. The harmful effects of carbon monoxide on testis weight in rats observed by Williams and Smith (1935) are also suggestive in that connection. According to Myers, Vidgoff, and Hunter (1933) dried bull testis-tissue fed to rats in 0.2-gram doses five times weekly for 85 to 117 days causes marked

atrophy of the testes, prostate, and seminal vesicles, and finally aspermia. These effects cannot be due to the androgenic activity of the lipoid fraction from bull testis-tissue because the accessory sex-organs have also been injured. Compare these effects with those produced by Ito and Kon in puppies and young rats after 3 to 9 weeks treatment with a concentrate of urinary androgenic material. Here the atrophy was limited to the testes and thymus while the gonad accessories were hypertrophied. It is, nevertheless, possible that part of the injurious effect on the testes may be due to impurities in the urinary extract. Lastly, the atrophy of pigeons' and doves' testes as produced by Riddle et al. (1934) by prolactin also calls for an explanation.

c. *The effects of sex hormones on the physiological activity of the anterior pituitary as determined by implantation studies.* With the introduction of the hypophysis implantation method (Smith, 1927, 1929; Zondek and Aschheim, 1927; Smith and Engle, 1927) much fundamental information on the hypophyseal-gonad interrelationship was obtained. The observations which have a special bearing on the gonad control under discussion here in part confirm the more important conclusions arrived at by the other methods of approach.

From the studies of Meyer, Leonard, Hisaw and Martin (1930, 1931, 1932), Evans and Simpson (1929), Engle (1929), we know that the hypophyses of gonadectomized rats show greater gonadotropic activity than from non-castrates when implanted into immature rats and that the injection of estrogenic material into the gonadectomized or normal rat lowers the gonadotropic effect by the implantation method. So also the anterior lobe transplants from the cryptorchid rat produce greater gonad stimulation. From these studies we also conclude that the female hypophysis is more easily depressed by "estrin" than the male pituitary. Other sex differences are also found as elsewhere indicated in this review. Quantitative studies on the effects of androgens in lowering the gonadotropic activities as measured by the implantation method have not been conducted.

d. *The testis control of the anterior pituitary as revealed by parabiosis studies.* In 1921 Matsuyama and Yatsu independently showed that parabiosis of a normal female rat with a castrated male leads to marked ovarian and uterine hypertrophy with large ovarian follicles and considerable luteinization. Goto (1925), Fels (1929) and Kallas (1929) confirmed these observations. Goto attributed the stimulation of the female sex organs as due to a hypothetical "kastrohormone" received from the blood of the castrated male. Kallas had the advantage of the

discoveries of Zondek and Aschheim (1927, 1928) and Smith and Engle (1926, 1927). Hence he concluded that the gonad-stimulating substance must be of hypophyseal origin. Kallas also showed that parabiosis of a normal female rat with a spayed rat leads to estrus in the normal female and that if an anterior pituitary gonad-stimulating preparation is injected into the spayed animal it hastens the appearance of estrus in the normal mate. Since estrus never occurs in the spayed mate, the conclusion is that estrogenic material does not pass over in sufficient concentration to influence the accessory sex organs of the castrate. The present day interpretation is that the concentration of ovarian hormone in the castrated animal is not sufficient to inhibit or control the gonad-stimulating activity of the hypophysis but that the gonad-stimulating hormone from the castrate does pass into the normal mate.

This type of experimental animal has been employed in attempting to determine the nature of the substance or substances of gonad origin which normally control the gonad-stimulating activity of the hypophysis. Martins and Rocha (1929, 1930, 1931) report results therewith which suggest a certain degree of sex specificity in the hypophysis. They found that if the spayed female united with a normal female is injected every other day with testis pulp, there is no change in the estrus and corpora lutea formation in the normal animal. However, if a castrated male is parabiotically united with a normal female and the male is then injected with the testis-tissue pulp, the estrus is delayed. Martins showed that a rat with cryptorchid testes also produces estrus in the normal female mate. Since the anterior pituitary of the cryptorchid rats contains castration cells, but the seminal vesicles appear normal and the testes are free from spermatozoa, Martins concludes that the inhibiting agent normally cannot come from the interstitial cells but must come from the spermatogenic elements. The assumption that cryptorchidism does not influence the accessory sex organs is, however, not correct because Nelson (1934) has now shown that if such animals are observed long enough the seminal vesicles and prostate also show castration effects. Nelson points out that the hypophysis is the most sensitive to changes in male-hormone concentration, but that seminal vesicles and prostate can be maintained at lower levels, hence cryptorchidism leads to the hypophyseal changes first. Martins and Rocha also observed a sex difference in the type of gonad-stimulating effect produced in the normal female when joined parabiotically with a castrated male or spayed female. The castrated male mate led primarily to follicle formation but the spayed female union usually led to

more luteinization. It is of some interest to note that if the castrated male infantile rat is donor to an infantile normal female, we also observe the estrus phenomena. Obviously, the infantile male pituitary normally is inhibited by a substance which originates in the testis tissue, but which has not affected the accessory male sex organs. In other words, if testosterone or androsterone are responsible for the normal control of the hypophysis, the concentration must be so low that it does not act on the accessories. It will be recalled that the reverse is true for the adult rat, that is, it requires only about one-fourth capon unit daily to affect the seminal vesicles and prostate in the castrated rat, but over ten capon units daily to control the anterior pituitary. Martins and Rocha with "an intensive treatment of castrated males with testicular aqueous extracts . . . succeeded in keeping the hypophysis in normal condition and without influencing the accessory sex glands." These findings are exceedingly important because if fully confirmed it means that the substance involved is neither androsterone nor testosterone. Whether it is theelin, theelol, or dihydrotheelin, remains to be determined. It is, however, quite probable that it is not one of the estrogenic substances. McCullagh and Walsh (1935) have confirmed Martins and Rocha in part. They find that the anterior pituitary activity in the castrated mate of the parabiotic pair can be inhibited by the lipin-soluble male-hormone concentrate and also by "testis mush." However, with the lipin material it requires the equivalent of very much larger amounts of tissue than with the "testis mush." Furthermore, the former regenerates the seminal vesicle and prostate in the castrate while the "testis mush" has no such effect.

Witschi, Levine, and Hill (1932) show that when a male rat, which has been sterilized by x-ray one month previously, is joined parabiotically with a normal female it leads to continuous estrus. In spite of this hypophyseal overactivity as a result of loss of a testis hormone, the testes were 25 per cent heavier than normal and the accessory sex organs were distinctly enlarged. The testes were free from germ cells. This could be interpreted as a case where the greater gonad-stimulating activity of the anterior pituitary is manifested not only on the normal female but also on the sterilized male. Since the hypophysis had become more active as a result of the destruction of the germinal cells, the conclusion is that a testis control of hypophyseal activity resides in the germinative tissue. The radium sterilization of male rats which Moltrom and Cramer (1923) also found to produce "castration changes" in the hypophysis and which led to a great increase in the number of

interstitial cells in the testis may be considered an earlier confirmation of the x-ray effects.

General conclusions on the testis control of the hypophysis. That the known testicular and urinary male-hormone preparations can inhibit the hypophysis gonad-stimulating activity is generally granted. However, the dosage involved is tremendous and at least five times as high for the male as for the female rat. Estrogenic substances on the basis of weight are more potent, but the dose required for males is also approximately five times that necessary for females. Considerable evidence indicates that another substance is elaborated by the testis tissue. This hypothetical substance appears to be active in the immature animal, but does not act on the accessory sex organs. In the control of the anterior pituitary lobe it appears to be more effective or else higher concentrations thereof exist in the testis tissue than of the known androgens. The substance seems to disappear from the testis with destruction of the seminiferous elements.

V. *More involved and less definitely established effects.* a. *Creatine and creatinine excretion.* The well known differences in creatine and creatinine excretion in relation to sex and puberty naturally suggested attempts at the control of creatine excretion by means of male-hormone therapy. Bühler (1933) concludes from the use of doses of 50 to 150 capon units of "Proviron" in creatinuria associated with lowered sexual activity in man that the male hormone acts specifically in the male in lowering creatinuria, that it inhibits creatine metabolism in women and is without action before puberty. The experimental evidence given is not as convincing as one might desire. Kun and Peczenik (1935) report that post-puberally castrated rats develop a creatinuria if 20 cc. of milk (containing about 0.4 mgm. creatine) are given by mouth. Normal male rats do not develop creatinuria on this milk feeding. The creatinuria developed in the castrates varies in different rats and for the same rat from day to day. The creatine content in urine varies from 10 to 55 per cent of the total creatinine plus creatine. The injection of 25 capon units of Proviron (probably a urine concentrate) actually increased the creatine excretion, but 30 to 125 capon units reduced the creatine content to a range of 0 to 26 per cent. Normal female rats show the highest creatine excretion during the estrous period and in ovariectomized rats theelin injection (500 to 12,000 mouse units) increased the creatinuria. In castrate males, theelin did not reduce the creatinuria. Steinach, Kun, and Peczenik (1936) attempt to classify cases of male-hormone deficiency in man on the basis of the degree of

creatinuria observed as a result of a uniform intake of creatine from a diet of hard cheese and dark meat. They conclude that true male-hormone deficiency exists only when amounts of creatine greater than 10 per cent of the total creatinine plus creatine are found in the urine. In the cases of "true male-hormone deficiency" this value varied from 10 to 27 per cent while in the "non-deficient" group the range was 0 to 9 per cent although the latter group were recognized as suffering with a weak libido. They do not give any data on the changes in creatine excretion as a result of androsterone benzoate therapy but state that the "non-deficient" group was not benefited otherwise whereas the "true male-hormone deficiency" cases were benefited as determined by other criteria. Among these criteria are such subjective findings as increase in muscular strength, less tendency to fatigue after physical or mental exertion, improvement in initiative and mental concentration with less sleeplessness and less pain in the extremities! The 5-mgm. doses of androsterone benzoate used by these authors represent approximately 50 international capon units. In other words, they actually used smaller amounts than the maximum doses used on rats. The reviewer feels that the interpretation or evaluation of analytical data on creatine and creatinine excretion are exceedingly uncertain on account of the difficulties involved in the analytical methods. Slight differences in the absolute excretion values are of very doubtful significance. Hence Steinach et al.'s attempts to classify types of male-hormone deficiency on such differences do not seem very well founded. The relation of creatine and creatinine metabolism to sex-hormone therapy should, however, be followed more intensively.

Kochakian and Murlin (1935) find that the castrate dog does not excrete creatine if kept on a creatine-free diet, thus indicating that castration does not affect endogenous creatine metabolism, but that the castrate may not be able to fix exogenous creatine as well as the normal male.

b. *Cell permeability studies.* Duran-Reynals et al. (1929, 30, 32, 34) accidentally observed that extract of testis tissue in Ringer's solution diluted with an equal volume of glycerol, when injected, appears to stimulate cell division locally. It also produces an increased permeability of the tissues as shown by the extent of the area containing India ink which was suspended in the extract. This activity is heat labile. It is precipitated *in vitro* by antisera against homologous testis extracts but no antagonistic action is produced *in vivo*. Although other organs and tissues contain the same activity, the testis is the most potent source

found thus far. McClean et al. (1931, 33) find protamine salts and extracts of spermatozoa to produce the same effect on tissue permeability. McClean (1930) finds the extract to enhance the local lesions following intradermal injections of vaccine virus and diphtheria toxin. Pijoan (1931) and Terada (1934) also confirmed the original observation of Duran-Reynals in connection with the enhanced and spreading effects of the testis extract on the infectious activity of organisms introduced with the extract. The exact nature of the substance involved has not been determined, but it is obvious that it is not of lipoid character. It is heat labile in aqueous solution, but when precipitated by alcohol and ether, it can be heated in the dry form at 100°C. for 30 minutes without destroying the activity. Incubation with trypsin destroys it.

c. *Blood coagulation.* Differences in rate of coagulation of blood have frequently been associated with sex. Bablik (1935) briefly summarizes the evidence that castration in rabbits and women results in slower coagulation of blood, the treatment of male dogs with "folliculin" hastens the rate of coagulation of blood and that in amenorrhea and menopause, the blood coagulates more slowly. On the basis of very few experiments by himself on gonadectomized rabbits of both sexes, he concludes that the administration of either of two proprietary male-hormone concentrates lengthens the time of coagulation of the blood. "Menformon" had the opposite effect.

d. *Composition of tissue proteins.* Tadokoro (1927, 1930, 1931, 1933, 1934) claims to find many fundamental biochemical differences in the sexes. He emphasizes in particular the chemical and physico-chemical differences of the muscle and skin proteins in the sexes. He reports that the greatest differences in the composition of the proteins reside in their histidine, arginine, and cystine content. He must consider a male sex-hormone factor to control the quantitative composition of the proteins because he claims that the composition of the myosin and myogen fractions from the capon muscle-tissue two months after castration approach the composition of the corresponding muscle-proteins from the hen. He reports similar differences in the composition of hide proteins from cow and bull and the muscle proteins in male dog and the gonadectomized litter-mates. The data as presented by Tadokoro are by no means convincing.

e. *Sex difference in resistance to drugs.* Lee (1935) makes equally dubious claims on the basis of very poor experimental evidence. He claims that in mice and rabbits, there is a sex difference in the resistance to nicotine poisoning, the females being more resistant. His evidence

for this claim is very unsatisfactory but he concludes, nevertheless, on the basis of very limited studies, that luteoglandol (Roche) increases the resistance of spayed mice to nicotine.

Very good evidence of a male-hormone control of resistance to drugs has been obtained by Holck and Kanân (1936). They find the female albino rat more sensitive than the male to barbiturates of the type evipal, pernoston, and amytal, but not to barbital. This difference in sensitivity is measured by the duration of hypnosis and the rate of acute mortality. Ovariotomy does not produce an appreciable change but castration of the adult male leads to a marked prolongation of the hypnosis and increases the rate of acute mortality to evipal, pernoston, and amytal, but not to barbital. Prolonged treatment with 10 to 20 capon units of male-hormone concentrates (also pure androsterone) per day decreases the recovery time in the spayed females to about one-third for a given dosage of the barbiturate; in the castrated male, the treatment prevented most but not all of the castration effects. In both cases the beneficial action is lost completely some weeks after the treatment has ceased.

Possible modes of biological formation of sex hormones. The occurrence of dehydroisonandrosterone and androsterone in human urine, the probable occurrence of testosterone in human testes, the assumption that the estrogenic substance in male urine is theelin, the presence of progesterone and allopregnanolone in the corpus luteum, the presence of pregnanediol in pregnancy urine, and the structural relations between these substances and cholesterol and bile acids suggests possible modes of their formation in the organism.

The assumption appears to be made quite generally that cholesterol necessarily is the precursor of all of these substances. However, inasmuch as we know practically nothing about the precursors or metabolism of cholesterol, it probably is just as likely that some of the sex hormones may be synthesized quite directly without passing through cholesterol. Whether carotinoids, vitamin A, squalenes, vitamin E, fatty acids, or carbohydrates are involved in these syntheses remains to be determined.

If we assume that cholesterol is the precursor, then the natural derivation of various sex hormones and related substances appears to be a relatively simple process on paper. These relations are shown on pages 191 and 192.

On page 192 the importance of cholestenone as a common hypothetical intermediate between cholesterol and the four substances cis- and trans-

dihydrocholesterol, progesterone, and Δ^4-androstenedione, is obvious. The fact that progesterone is accompanied by allopregnanolone (Butenandt and Mamali, 1935) in corpus-luteum extracts and that pregnanediol and allopregnanediol (Hartmann and Locher, 1935) occur in pregnancy urine suggests that dihydrocholesterol may be the immediate precursor of allopregnanolone or that progesterone yields both allopregnanolone and pregnanolone and that the latter is reduced to pregnanediol as a waste product.

The relations of cholesterol, cholestenone, and androstenedione to androgens and to estrogens are indicated on page 192. The presence of dehydroisoandrosterone in human urine suggests its direct formation from cholesterol by specific oxidation of the long side-chain in position 17. Testosterone might then be formed from it with androstenediol as an intermediate. However, testosterone could be formed quite as easily from cholestenone with androstenedione as an intermediate. In either case the reduction of the 17-keto group to a secondary alcohol makes probable the formation of two isomers if carried out *in vitro*; biologically these reactions are usually controlled to produce only one form. As a result of the very recent work of Ruzicka and Kägi (1936), we now conclude that the natural form of testosterone contains the OH group in the 17-trans position because these investigators were able to obtain two forms of testosterone which involved the configuration of carbon atom 17. They separated the two forms and found the trans form approximately thirty times as potent as the cis form by the capon test. In rats, the cis form produced no reactions in the seminal vesicle and prostate in doses forty times that of the trans form. From these and related studies they now conclude that an OH group in the cis position on carbon 17 of the cholane or cholene ring lowers the potency very strikingly.

Androsterone calls for very specific configurations on carbons 3 and 5. This could be formed *a*, from testosterone by reduction of the 3-keto group, hydrogenation of the 4,5 position and oxidation of the 17-hydroxy group; *b*, from androstenedione by simply reducing the 3-keto position and hydrogenating the 4,5 position; *c*, from a hypothetical specific intermediate androstanedione by simply reducing the 3-keto group. In all of these reactions, isoandrosterone could also be formed in equal amounts but biological processes usually produce only one such isomer selectively. However, androsterone may also be derived from cholestenone, with epidihydrocholesterol as an intermediate. If we can assume that the species which secrete male sex-hormones all have

the same one, testosterone, just as adrenalin is found in all adrenals, then it would seem more likely that if cholesterol is the precursor we should have the degradation go by way of cholestenone and androstenedione or dehydroisoandrosterone. The formation of androsterone from testosterone appears more probable than from epidihydrocholesterol.

The assumption of the formation of the estrogens from androstenedione or from testosterone is more far fetched. In fact there is no direct evidence that such is the origin of theelin and the related estrogens. These processes all call for the specific removal of the methyl group in position 10 and for the production of various degrees of unsaturation of rings A and B. It appears much more likely that these unsaturated rings are built up more directly from precursors other than cholestenone.

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ADDENDUM. *The modification of sex development in the chick embryo by male sex-hormones.* Studies by Willier, Gallagher and Koch (1935, 1937) show the effects of injection of high concentrations of androgens into incubating hens' eggs on the reproductive system of the 18- to 19-day-old embryo. Bull testis-tissue concentrates in doses of 15 to 300 international capon units per egg had no observable effect on the gonads or oviducts of either sex; the Wolffian ducts (potential male ducts) may have been slightly hypertrophied. Human-urine concentrates, freed from phenolic estrogens, in doses of 12 to 24 international capon units per egg, at times modified the left testis in the males into an ovotestis and produced an ovarian cortex in the right gonad of the females; the Wolffian ducts were consistently, tremendously swollen but the oviducts were rudimentary or absent in the female embryos. The results on the female with urinary concentrates are in part similar to those produced by pure theelin and theelol. The different effects produced by the two types of concentrates may possibly be correlated with the estrogenic-like action observed by some with androsterone and dehydro-androsterone. Undoubtedly the concentrates also contained toxic substances which caused death in many of the embryos.

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RODS, CONES, AND THE CHEMICAL BASIS OF VISION

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I. In the last few years there has accumulated a considerable amount of new and highly precise data describing various visual functions. In addition, many of the older measurements have been freshly evaluated, and the whole field of vision has achieved a quantitative form not recognized a generation ago. The first purpose of the present paper is to bring together these recent measurements and evaluations, and to show how easily and strikingly they fall into the pattern of the Duplicity Theory which separates vertebrate vision into cone function and rod function.

The value of the material is by no means exhausted by this treatment. Rather, it is enhanced because of the identification of specific measurements with known morphological units, whose chemical properties may then be investigated. The second purpose of this summary is to deal with the quantitative aspects of the data as they bear on the possible chemical systems present in the retinal receptor elements.

II. Duplicity theory. The retinas of most vertebrates contain two different types of receptors,—cones and rods; and this fact has led to the notion that the vertebrate retina is not one sense organ, but two.

Consider the human eye. Structurally the center of the retina is occupied exclusively by cones, while the rest of the retina contains rods and cones, with rods increasingly predominant toward the periphery. Since at high illuminations vision is most efficient with the center of the retina, whereas at low illuminations it is most effective with the periphery, the idea of a double visual organ associates the cones with vision at high light intensities, and the rods with vision at low light intensities. Moreover, since we see color best at high intensities and see no color at low intensities, we may consider the cones as specific receptors for color, and the rods as the general receptors for light regardless of color.

In essentially this form the idea for a retinal double sense organ was proposed by Max Schultze in 1866 on the basis of the histological studies which he made of the vertebrate retina, coupled with the physiological

knowledge contributed by his contemporaries Aubert (1865) and Helmholtz (1866). However, no one seems to have paid any attention to the idea,—which is curious because Schultze was an important figure in his time, and his histological work was recognized and appreciated. The neglect of the concept was so complete that it is not even mentioned in the second edition of Helmholtz's *Physiological Optics*, which appeared in sections between 1884 and 1894, and was finally published as a whole in 1896. By that time the notion of a double retinal function had been independently suggested by Parinaud (1881) as the result of his observations on night-blindness and other pathological disturbances of vision, and by von Kries (1895) as an explanation of the many properties and ramifications of the Purkinje phenomenon (Purkinje, 1825; Dove, 1852).

The observations of Purkinje and later of Dove had shown that when widely different colors are matched in brightness at high intensities, the matches are not valid at low intensities, and the reverse. Parinaud (1884, 1898) had tried to explain the presence of this phenomenon in the visual periphery, as well as its absence in the fovea, in terms of visual purple. But it was von Kries' merit to have attributed it correctly to the different sensibilities of the rods and cones in the spectrum,—a concept which is still basic.

Since its reintroduction by von Kries and Parinaud, the idea of a double retinal function has been called the Duplicity Theory, and has persisted to the present, though not without the criticisms of Hering (1915) and Hess (for a summary see Hess, 1920) made largely as the result of experiments which we know now to have been erroneous (*cf.* especially Dieter, 1924; and Gross, 1928). With the years, the evidence for it has become more extensive and impressive; and when before his death von Kries (1929) summarized its status, he could feel certain that the double function of the retina in terms of rods and cones was a well-established concept in physiology.

The summary which von Kries wrote rests in the main on the roughly quantitative implications of the Purkinje phenomenon. To a certain extent von Kries was unaware of the later developments in vision, and did not include them in his treatment. But even since 1929 the newer data which have become available are not only more plentiful than those known to von Kries, but possess a much higher precision and definitiveness. For them also the Duplicity idea serves as a simple reference frame.

III. Spectral sensibility. a. *Low intensity and rods.* The oldest

physiological difference recognized between rods and cones depends on the relative sensibility of the eye to the spectrum at low and at high intensities. We shall consider this first as it relates to the rods. When the visible spectrum is reduced to an intensity near the threshold and is viewed by a dark-adapted eye, it appears colorless. Beginning perhaps with Ebert (1888) and Langley (1888), a number of investigators (Hillebrand, 1889; Koenig and Ritter, 1891; Abney and Festing, 1893; von Kries and Nagel, 1896; Schaternikoff, 1902; Pflüger, 1902; and Trendelenburg, 1904) studied the relation between wavelength and the amount of light required to produce a specific brightness at or near the threshold.

The main difficulty with this early work was that the energy distribution in the spectrum was not known, and therefore the relative effectiveness of the spectrum was dependent on the particular source of light used. The best of the measurements were by Koenig, who corrected his data for spectral energy distribution in terms of Langley's measurements of sunlight. Koenig found that no matter what the other visual characteristics of his various subjects were, their maximum visibility at low brightnesses was at about 500 mμ.

Thirty years after Koenig, Williams and I (Hecht and Williams, 1922) reinvestigated the relation with modern methods making our own energy measurements, and using 48 normal individuals in order to get an average function. Later Kohlrausch (1923), Laurens (1924), and Sloan (1928) repeated the measurements with one observer each, and agreed with us in placing the maximum effectiveness of the low intensity spectrum at very nearly 510 mμ. Our average data are shown in the lower portion of figure 1. They give the relative energy required at the different wavelengths for the eye to see a light slightly above the threshold after an hour of dark adaptation. There can be little doubt that these data represent a function of the rods. At such low intensities the fovea, containing a predominance of cones, is completely blind; and the measurements are best made with large fields falling well in the periphery of the retina.

The effectiveness of the spectrum at low intensities has just been determined for two other mammals. Graham and Riggs (1935) measured it with the white rat, using the retinal potential as an index; and Brown (1936) has examined it for the rabbit, using a conditioned respiratory reflex. In both cases the spectral effectiveness is almost identical with what Williams and I found for the human eye. The white rat's spectral sensibility is the same at high and low intensities; its vision is thus

entirely a rod function. This corresponds to the histological demonstration that its retina contains only rods and no cones, as well as no central region of compactly grouped elements (see especially Lashley, 1932).

It is obvious that human eyes which lack color vision completely, and therefore possess no cone function, should resemble the white rat's

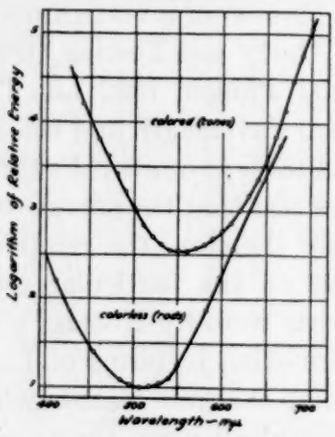

Fig. 1

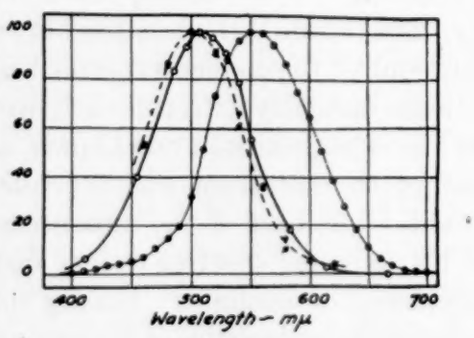

Fig. 2

Fig. 1. Relation between wavelength and relative energy required to produce a specific visual effect at high and at low illuminations. The lower data are from Hecht and Williams (1922) and represent the relative energy required to produce a barely perceptible brightness after prolonged dark adaptation. The upper data are from Hyde, Forsythe and Cady (1918) and give the relative energy to produce a given high brightness, using only the fovea. The two curves are each accurately drawn from their separate data. Their vertical separation, however, has been arbitrarily arranged so that they are nearly coincident at the red end of the spectrum; this is a graphic expression of the fact that the colorless and color thresholds of the eye are nearly identical in the red.

Fig. 2. Visibility curves and the absorption spectrum of visual purple. The empty circles and continuous line record the relative effectiveness of the spectrum at the lowest intensities; they are the reciprocals of the energies in figure 1 so arranged that the maximum effectiveness is 100. The filled circles and those with a line through them are the absorption coefficients of visual purple of the rabbit and monkey respectively also computed for a maximum of 100; the measurements are from Koettgen and Abelsdorff (1896). The half filled circles and dotted line are the average data of Gibson and Tyndall (1923) for the visibility of the spectrum at high intensities by persons of completely normal color vision.

eye in this respect and should show the low intensity spectral effectiveness at all intensities. Measurements with a complete colorblind were first made by Donders (1882) and soon after by Koenig and Dieterici (1886). After Hillebrand's (1889) measurements at low intensities, it was apparent that the colorblind data, though secured at ordinary intensities, resembled the normal low intensity data secured with a simi-

lar spectrum. This was confirmed by Hering (1891), by Koenig and Ritter (1891), later by May (1907) among others, and most recently by Geldard (1933). Geldard was the first actually to determine the energy distribution in the spectrum used for measurements with a complete colorblind, and he found that even though the spectral sensibility measurements were made at moderately bright intensities, the resulting distribution practically duplicated our 1922 measurements at threshold intensities shown in figure 1.

b. *Visual purple.* The most interesting property of the lower curve in figure 1 is its relation to the absorption spectrum of visual purple, the photosensitive pigment in the outer segments of the rods. Visual purple was discovered by Boll (1876) and extracted from the retina by Kuehne (1879) who determined many of its properties. Since then it has been investigated by Koenig (1894), by Koettgen and Abelsdorff (1896), and by Trendelenburg (1904, 1911), who were all mainly concerned with its absorption spectrum; by Garten (1906, 1907) and by Hosoya and Bayerl (1933) and by Hosoya (1933), who dealt with the changes in the absorption spectrum during and after bleaching; by Hecht and Chase (1934) and by Chase (1936), who have partially cleared up the conflicting reports in the work of previous investigators; by myself (Hecht, 1920a, b; 1924b), and by Weigert and Nakashima (1929, 1930), who studied certain aspects of its photochemistry; and by Wald (1933; 1934; 1935a; b; 1936), who has given important information about its chemical constitution.

If visual purple is the photosensitive material responsible for rod vision, then its absorption spectrum should be intimately related to the spectral sensibility of the eye at low intensities. To make the comparison, it has to be assumed that a just perceptible visual effect requires the absorption of a constant amount of energy, I_a, regardless of wavelength. Strictly, the assumption should be that a constant number of quanta is required, but because of the small range of wavelengths the difference between the two is negligible. If I is the incident energy, a the concentration of visual purple, and ϵ the absorption coefficient, then

$$I_a = I\,(1 - e^{-\epsilon a}) \tag{1}$$

in terms of Beer's law. The exponential expands into the series

$$e^{-\epsilon a} = 1 - \epsilon a + \frac{\epsilon^2 a^2}{1 \cdot 2} \cdot \cdots \tag{2}$$

in which terms having powers higher than unity may be neglected be-

cause only threshold effects and small concentrations are involved. Substituting in (1) and rearranging, we get

$$\epsilon = I_a/I\,a \qquad (3)$$

which puts the absorption coefficient as inversely proportional to the incident energy required for a threshold effect.

Koenig (1894) first made this comparison. His visibility data, corrected approximately for energy distribution in the spectrum, resemble the absorption data of visual purple secured from a single human eye, though the two maxima seem to differ by about 5 mμ. Later, Trendelenburg (1904, 1911) showed that the rate of bleaching of visual purple follows its absorption spectrum, and is closely related to the low intensity visibility curve as well. Trendelenburg made no measurements of spectral energy distribution; his results, approximately corrected for energy by Henri and Larguier des Bancels (1911), roughly resemble Koenig's findings.

An adequate comparison between the visibility curve and the absorption spectrum of visual purple was made possible only when the accurate data of figure 1 became available. Figure 2 shows this comparison between *a*, our data plotted as visibility, that is, the reciprocal of the required energy, and *b*, the absorption spectrum of monkey and of rabbit visual purple as measured by Koettgen and Abelsdorff (1896). The data of Koettgen and Abelsdorff are the most complete; however, because of the method used, their determinations at 440 and 420 mμ are probably in error, and have been omitted (*cf.* Garten, 1907; Chase, 1936).

Figure 2 shows that the two sets of data are almost identical but that the visibility curve is displaced about 7 mμ toward the red. This difference appears even in the older data of Koenig and of Trendelenburg. Since the low intensity visibility curves of the rabbit and of the white rat are practically identical with the one for man, this displacement relative to the absorption spectrum is present also for them. Moreover, the work of Chaffee and Hampson (1924) on the spectral sensibility of the frog's eye also shows a displacement of similar magnitude in relation to the absorption of frog visual purple as measured by Koettgen and Abelsdorff.

This displacement may be due to the difference in medium. Measured with a spectrophotometer, visual purple is in aqueous solution, whereas in the retina visual purple is in the dense and highly refractive outer segments of the rods. In terms of Kundt's rule (Kundt, 1878) the shift is in the right direction, that is, the absorption maximum moves

toward the red as the density and refractive index of the medium increase (*cf.* especially Becking and Koning, 1934). Therefore we may conclude that the dim visibility curves of the human eye, the rat eye, the rabbit eye, and the frog eye, correspond to the absorption spectrum of visual purple in the condition in which it is present in the terminal segments of the rods.

An additional bit of proof comes from the fact that fresh water fishes have a visual purple whose maximum is not at 500 mμ but at 540 mμ (Koettgen and Abelsdorff), though marine fishes seem to fall between these two values (Bayliss, Lythgoe, and Tansley, 1936). The visual purple of the sunfish *Lepomis* has its maximum absorption at 540 mμ, and Grundfest (1932a) has found that the maximum of its low intensity visibility curve also comes at nearly the same place.

c. *High intensities.* The visibility of the spectrum at high brightnesses is usually found by measuring the relative energy at different wavelengths required to match a high constant brightness. Such measurements date from Fraunhöfer (1814), who used the relative brightness in the spectrum as a substitute for the then unknown energy distribution. With the introduction of energy measurements by Langley, the visual effectiveness of the spectrum at high intensities has been repeatedly determined.

The best of the earlier measurements were made by Koenig and Ritter (1891) who compared by direct photometry different parts of the spectrum with one portion of it. This is a fairly brutal method; and since then, various tricks have been devised to avoid the subjective difficulties of such heterochromic comparisons. Measurements have been made with many observers by Hyde, Forsythe, and Cady (1918), by Nutting (1920), by Ives (1912b), and by Coblenz and Emerson (1918). Many other investigators, too numerous to record, measured the distribution for their own eyes, but in most cases, except those specifically mentioned, the energy distribution of the source was not known, and the data are useless as absolute statements of the relation between energy and wavelength.

Probably the most adequate data are by Gibson and Tyndall (1923), using heterochromic comparisons of small wavelength steps. The averages of some fifty color-normal individuals are in figure 2; the data are almost identical with the earlier measurements of Hyde, Forsythe and Cady, of Ives, and of Coblentz and Emerson. The maximum is at 555 mμ, which is 45 mμ farther toward the red than the low intensity visibility curve also shown in figure 2.

Anatomically, the high intensity measurements in figure 2 certainly

represent the behavior of cones, since the observations were made with small fields falling within the fovea which is composed mostly of cones. The brightness was quite high, with color vision dominating. However, even with low brightness and dark adaptation, cone behavior alone is apparent in visibility curves provided only small fields of about 1° are used (Sloan, 1928).

Precisely what the cone visibility curve represents is hard to say. Ultimately, of course, it must be the sum of the absorption spectra of the sensitive materials in the cones. At the present time it is generally supposed, in accordance with Young's (1807) idea of the basis for color vision, that there are three types of cones, each with its own sensitive substance. But there is no unanimity in the suggestions for the spectral sensibility distributions of the three receptors. Young supposed their absorption maxima to be widely different in the spectrum, and this supposition was adopted by Helmholtz (1866), by Koenig and Dieterici (1892), and has persisted up to the present (*cf.* Wright, 1934). Helmholtz later (1891) changed his mind and for very good reasons made the three absorption curves resemble one another; unfortunately his particular curves each have two maxima, and when added together do not even remotely resemble the visibility curve. I have shown (Hecht, 1930; 1931; 1932) that in order to describe with any precision the quantitative data of color vision, particularly those involving saturation, one must assume that the three cones possess absorption spectra which resemble one another closely rather than the reverse. Such absorption curves describe the data, and when added together reproduce the visibility curve exactly.

To settle the matter it would be well to isolate the cones, or to extract their sensitive materials, and to measure them. Unfortunately this has so far proved impossible. Even the evidence presented by von Studnitz (1932) to prove that the cone retina as a whole shows decreased absorption after exposure to light is rather dubious; photomechanical changes in such retinas are known to occur (Garten, 1907; Arey, 1915; Detwiler, 1916), and these would be sure to influence the transmission of a semi-opaque tissue like the retina.

d. *Other animals.* Other vertebrates which, like man, also possess both cones and rods can be expected to show two types of visibility curves, one at low intensities and another at high intensities. Such an expectation was first confirmed by Himstedt and Nagel (1900) and in greater detail by Piper (1904; 1905a) who found two spectral sensibility curves for the frog's eye by measuring the retinal currents produced on

illumination (*cf.* however, Chaffee and Hampson, 1924). Moreover Piper (1905a) found that day birds such as the domestic hen and the pigeon show only one spectral sensibility distribution which corresponds to the high-intensity cone visibility curve of man; while night birds like the owl, and mammals like the dog, cat, and rabbit also show only one sensibility curve corresponding in this instance to the rod visibility curve of man.

Fish are known to have rods and cones (Schultze, 1866; Wunder, 1925); and Bauer (1910, 1911) and von Frisch (1925) found that they show the Purkinje phenomenon, indicating that the high and low intensity visibility curves differ in position on the spectrum. Grundfest (1932b) actually determined the two curves for the sunfish *Lepomis* and found the low intensity maximum at 540 mμ and the high intensity maximum 30 to 60 mμ farther in the red. The average shift between the two maxima is about the same as for the human eye and for the frog eye (Himstedt and Nagel, 1900; *cf.* especially Kohlrausch, 1931a).

e. *Consequences.* At low intensities the visibility curve is determined by the rods because the cones do not function, while at high intensities it is given by the cones because of their numerical preponderance in the fovea (*cf.* Abney and Festing, 1891; Rosenberg, 1928). What will happen at intermediate intensities in retinal locations having rods and cones depends on whether the contributions from the two receptor systems remain separate or are fused in the resulting sensory effect.

With brightness the situation is simple. Koenig and Ritter (1891) were able to show for the human eye that the relative brightness of the spectrum at different intensities passes gradually from one system to the other, and that at some intermediate intensities the visibility of the spectrum actually shows two maxima (*cf.* also Sloan, 1928; Forbes, 1929). Clearly, brightness is a function in which the two retinal systems pool their effects additively.

There are other functions in which the two systems behave more independently, and in which the transition from one to the other is fairly sharp. In order to understand the behavior of such functions, it is well to consider the visibility curves again, but from a slightly different angle. The two curves are shown in figure 1 in the form of relative energy for a constant brightness against wavelength. Each curve is accurate by itself, but the vertical separation between the two is arbitrary because its exact extent varies somewhat with the position on the retina.

Beginning at the lowest energy level and moving vertically along any

ordinate, light produces its first visual effect when its magnitude reaches the rod curve. The particular function continues to be controlled by the rods as the energy increases, until the cone curve is reached. At this point if cone activity is independently recognized, a definite change in the character of the function should become apparent, which will be maintained as the intensity increases. The significant feature of figure 1 is that because of the different minima of the curves, this rod-cone interval must vary with the wavelength, being very small in the red, and large in the blue.

A case in point is the sensation of color, characteristic of cone function. The intensity range between rod threshold and cone threshold in figure 1 is controlled by rod function and appears colorless. Above the cone curve, color makes its appearance. The rod-cone, or colorless-to-color, interval should then be small in the red and large in the blue. This is indeed the fact, as shown by the work of Charpentier (1880) and Parinaud (1898) who refer to the difference between rod and cone thresholds as the photochromatic interval.

In respect to intensity and spectrum, all the visual phenomena to be described show a sufficient degree of independence in rod and cone function for their effects to be easily differentiated and understood in terms of figure 1.

IV. Dark adaptation. a. *Rods and cones.* The capacity of the human eye to increase in sensibility during a stay in the dark was first described by Aubert (1865). Since then, this phenomenon has been frequently investigated in man and other animals (for summaries see Adams, 1929; Müller, 1932; and Hecht, 1934a). For the human eye, the data fall quite neatly into what may be expected in terms of rod and cone function.

The earliest measurements were by Piper (1903) and were confirmed by Nagel (1911) and by others since. The determinations were made with white light, large measuring fields, and peripheral observation. In general, the measurements show a continuous decrease in threshold extending for about 30 minutes and covering an intensity range of about 5000 to 1 units of intensity.

This change was correctly ascribed to the rods because of its location in the retinal periphery and because of the lack of color at the threshold. In fact, it became generally accepted that the rods alone could dark adapt while the cones could not, though there was some evidence (Nagel and Schaefer, 1904; Inouye and Oinuma, 1911; Dittler and Koike, 1912) to show that the cones do adapt slightly.

The matter was finally settled when the dark adaptation of the rod-free area of the fovea was accurately measured (Hecht, 1921), and the reasons for the previous failures became apparent. These measurements were confirmed next year by Kohlrausch (1922) and later by Graham (1930). Figure 3 shows the data, from which it is apparent that foveal dark adaptation is very rapid and practically over in 3 minutes. It is precisely the speed of cone adaptation which delayed its discovery, because the technical procedure used by Piper and by Nagel and Schaefer and the others was so slow that they missed the major part of the phenomenon almost completely.

If both cones and rods show dark adaptation, then measurements of the eye as a whole should show both phenomena. This was first demonstrated by Kohlrausch (1922) who studied the dark adaptation

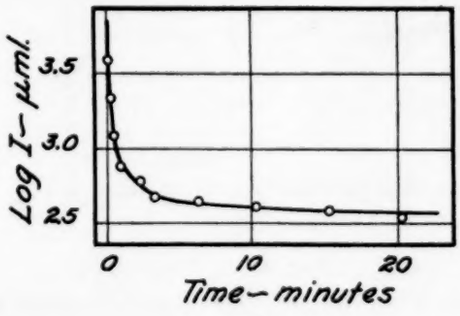

Fig. 3

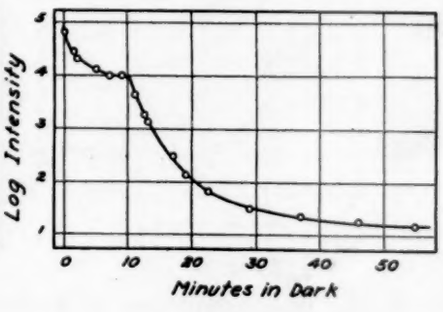

Fig. 4

Fig. 3. Dark adaptation of the fovea as measured with red light. The data are from Hecht (1921), and each point is the average of 30 measurements with 15 observers. Intensities are in micromillilamberts.

Fig. 4. Dark adaptation of the eye as a whole. Measurements of Engelmann's eye made by Piper (1903). Note the presence of cone adaptation and later of rod adaptation.

of a retinal area containing rods and cones, and found that its curve of dark adaptation shows a distinct break, preceding which adaptation is a function of the cones, and after which it is a function of the rods. It is interesting to record that on reëxamination, some of Piper's 1903 measurements, as well as some made later (Piper, 1905b), show this break strikingly, but that it was obscured by the absurd method of plotting adaptation then in vogue. For historical reasons, I give in figure 4 the data for Engelmann's eye taken from Piper's 1903 paper. It is apparent that the primary drop in threshold is rapidly over, as is the case with the foveal cone adaptation in figure 3, while the secondary drop in threshold appears later and proceeds for over 30 minutes.

b. *Separation by color and preadaptation.* If the primary and second-

ary adaptations apparent in figure 4 are really concerned with cone and rod function, it should be possible to isolate them to various extents by means of their visibility curves. Figure 1 shows that with extreme red light the final thresholds of the rods and cones are about the same. Therefore dark adaptation measured with red light will most likely record only cone adaptation since it is the more rapid. On the other hand with blue light, after completion of the rapid cone adaptation, the threshold should continue to drop because of the low level to which the rods finally sink. For colors between these, an intermediate situation is to be expected.

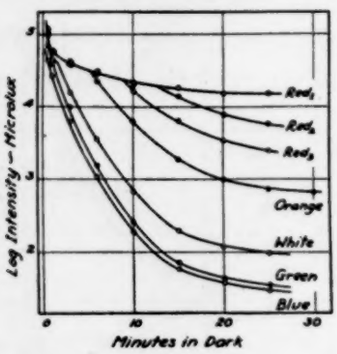

Fig. 5

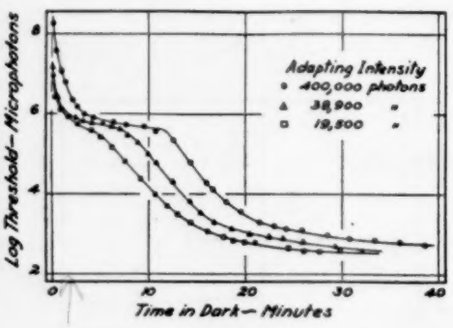

Fig. 6

Fig. 5. The dark adaptation of an area 1° in diameter, situated 5° above the fovea (Kohlrausch, 1922; 1931b). Red_1 is the only color which limits the measurements to the cones. The other two reds let through more orange light, and show up rod adaptation. With blue light, cone adaptation is barely evident. *Cf.* figure 6.

Fig. 6. The dark adaptation of a 5° field situated 30° nasally (Hecht and Haig, 1936). Though measured with extreme violet light, the course of adaptation shows two distinct sections, due to the high light adaptation preceding the measurements. Note that the secondary, rod adaptation appears later the higher the intensity of preadaptation.

Essentially in terms of these ideas, Kohlrausch (1922, 1931b) investigated the dark adaptation of a small retinal area 5° from the center, using lights of different color. His data are shown in figure 5. It is apparent that for most colors there are two very distinct parts to dark adaptation. The first part corresponds to cone adaptation, and is nearly the same for all the colors which show it. Like the adaptation in figure 3, it is over in about 3 minutes. Soon, however, rod dark adaptation begins to show and, depending on the photochromatic interval, it appears sooner and goes lower as the light moves to the blue.

Kohlrausch's measurements with blue and green light show hardly

any cone adaptation. This is purely because his preliminary light adaptation was not high enough in intensity to bring it out. Haig and I (Hecht and Haig, 1936) have recently studied this aspect, and find that even when measured with extreme violet light, cone adaptation is strikingly apparent, provided the light adaptation is intense enough. Our measurements with three different intensities of light adaptation are shown in figure 6. It is clear that the higher the light adaptation the more prominent is the cone adaptation, and the later does the rod adaptation appear.

Figure 6 is significant in demonstrating the appropriateness of describing these data in terms of cones and rods. The filled-in symbols in the figure indicate that a violet color is apparent at the threshold during the measurements; if the intensity is reduced below the particular value indicated, light and color disappear together. The clear symbols indicate that no color can be recognized at the threshold. Evidently, color is always associated with the primary, rapid adaptation which is attributed to cone function, while no color is apparent in the secondary, slow adaptation attributed to the rods.

The difference in the rates of cone and rod dark adaptation is associated with an inverse speed of light adaptation. Judging by the measurements of Müller (1931), the rods adapt to a given light intensity more rapidly than do the cones.

Müller measured the dark adaptation which follows the preadaptation of the eye to white light of 3000 lux for different durations. His data are shown in figure 7. After 1 minute of light adaptation, only the secondary, rod, dark adaptation is in evidence. Longer exposure to the preadapting light apparently changes neither the speed nor the extent of the rod adaptation, but merely delays its appearance. Cone dark adaptation, however, begins to show up only following a 2 minute preadaptation, and becomes more evident the longer the previous light adaptation. Evidently, because of the greater light sensitivity of the rods, they reach a photostationary state in the light sooner than do the cones. These results have very recently been confirmed and extended in a preliminary report by Wald and Clark (1936).

c. *Separation by retinal location.* It has long been established (Schultze, 1866; Rochon-Duvigneaud, 1907; Wolfrum, quoted by Dieter, 1924; Østerberg, 1935) that the histological structure of the human retina varies in different parts according to a definite pattern. There is a central area whose diameter is very nearly 1.5° which is completely rod-free, while a slightly larger area, 2° in diameter, contains so few rods

that it may be considered practically rod-free. Outside of this restricted central region, the retina contains rods and cones. Østerberg (1935) has shown that from the center outward the number of cones per unit area decreases at first rapidly and then more slowly, while the number of rods per unit area gradually increases out to about 18° and then slowly decreases again. For the greater part of the retina the ratio of rods to cones in an area of given size increases as the area is moved from center outward.

In terms of these histological facts, it follows from the Duplicity Theory that dark adaptation measurements with white light should

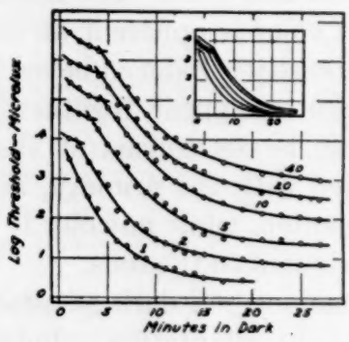

Fig. 7

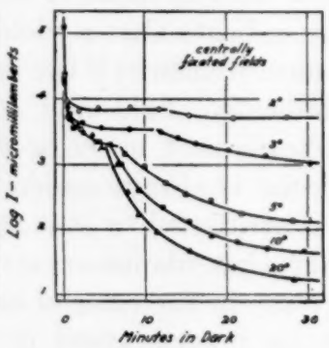

Fig. 8

Fig. 7. Dark adaptation of the eye after preadaptation for 1, 2, 5, 10, 20, and 40 minutes to 3000 lux (Müller, 1931). The time axis is the same for all the curves. The ordinates apply only to the lowest curve; for convenience, each series is displaced 0.5 log unit above the other. The inset brings the data together, and shows that the longer the preadaptation, the more evident is the primary cone dark adaptation, and the more delayed is the secondary rod adaptation.

Fig. 8. The threshold during dark adaptation for centrally fixated areas whose diameters are shown on the data. The larger the field, the sooner does the secondary rod section appear, and the larger is the intensity range covered by it. The data are from Hecht, Haig and Wald (1935).

show almost a pure cone behavior when restricted to the 2° central area, and a combination of both rod and cone behavior for larger central areas. We have recently published measurements (Hecht, Haig, and Wald, 1935) which bear out these predictions.

Figure 8 shows the data secured with a series of centrally fixated areas, using white light for measurements. With the 2° field, dark adaptation is mainly a foveal cone function, a slight secondary adaptation making itself apparent after about 15 minutes. For areas larger than 3°, the secondary rod adaptation shows itself unmistakably, the rod thresholds appearing sooner and adaptation going lower as the

area increases. Beyond 20° there is no very great change with increased area.

The primary cone portions of the curves for areas larger than 2° are not the same as for the 2° field. This is understandable because as the area increases there are brought into play not only rods but also cones outside the fovea. It is hardly to be expected that the thresholds and rates of adaptation of these peripheral cones are the same as those of the central cones.

There exists a well-established body of data describing the relation between the area of a field and the minimum intensity required to make it visible (for a summary see Parsons, 1914). At first glance, therefore, it might seem that the decrease in threshold which shows itself with the larger areas is due to the area-threshold relationship. However, it is simple to show that the major factor involved in these data of dark adaptation and final threshold has almost nothing to do with area but rather with the fact that the retina is not a uniformly sensitive surface. Coupled with its histological structure already referred to, it possesses a permanently graded sensitivity.

This may be best illustrated in figure 9 which shows the dark adaptation of a 2° field placed in different retinal positions. In the center at 0° off the central axis, adaptation is essentially cone and, as before, is rapidly over and limited in extent. Secured at $2\frac{1}{2}$° from the center, the adaptation curve shows the usual rod-cone dichotomy. The final threshold drops still more as the area is moved out to 5° and 10° from the center.

d. *Night-blindness.* Hemeralopia is a condition in which vision at low intensities is markedly poorer than normal, and usually becomes manifest to its possessor by his inability to see things in the dim lights at night. It has been known since ancient days; and from 1881 it has been associated with deficient rod vision (Parinaud, 1881; Treitel, 1885). There are evidently two varieties of night-blindness. One is temporary; it is caused by a vitamin A deficiency (Holm, 1925; Fridericia and Holm, 1925; Tansley, 1931), and may be cured with cod-liver oil. Its existence is understandable in terms of the connection between vitamin A and visual purple demonstrated by Wald (1936). The other variety is inherited (Oguchi's disease in Japan) and is apparently incurable. Probably some other constituent of rod function is missing in this case.

After Piper's first measurements of dark adaptation and their association with rod function, it was natural to think of testing the dark adaptation of night-blind individuals. This was first done by Hein-

richsdorff (1905) with Piper's apparatus. The conditions for making the determinations, especially the necessity for proper preadaptation to light, were not understood then, and Heinrichsdorff's data are not so obvious as those of Messmer (1908) in which hemeralopes clearly show a diminished dark adaptation; in some cases it is almost completely lacking.

Later, and apparently independently, Takagi and Kawakami (1924) measured the dark adaptation of several cases of hereditary night-blindness and found that for the first two hours in the dark these individuals showed almost no change in threshold, while under the same conditions of measurement the normal eye had dropped in threshold to about

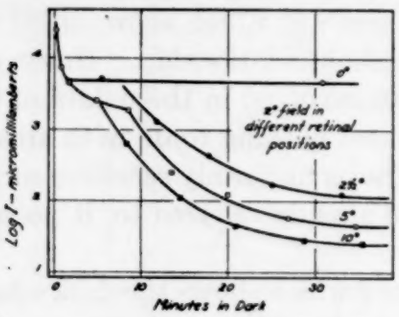

Fig. 9

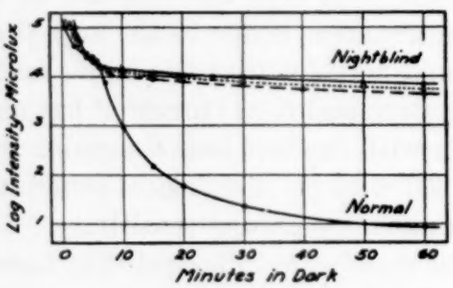

Fig. 10

Fig. 9. Dark adaptation as measured with a 2° field placed at the different distances from the center recorded on the data. Compare this with figure 8 for centrally fixated fields of different size. The measurements are from Hecht, Haig and Wald (1935). Note that the farther in the periphery the measurements are made, the sooner does the rod section appear, and the lower is the final threshold.

Fig. 10. Dark adaptation of three permanently nightblind individuals, compared with the normal. Data of Dieter (1929). Nightblind dark adaptation evidently shows only primary cone adaptation. Compare this with figure 3.

1/5000 of its initial value in 30 minutes. Rod adaptation seems to have been mainly lost, or at least greatly retarded in speed and reduced in extent. These findings were confirmed by Dieter (1929).

Dieter's measurements are shown in figure 10. They are chosen not because they demonstrate anything fundamentally different from the data of Messmer or of Takagi and Kawakami, but because they were very carefully made with modern apparatus and control and are therefore more precise as data. From them it is apparent that for the first hour at least, dark adaptation of hemeralopes shows only the primary cone drop in threshold and no secondary rod drop. Dieter's evidence is particularly conclusive because he found, under all conditions of brightness, that his hemeralopes possessed only one visibility curve, which

corresponded to his own high intensity, cone visibility curve. This natural experiment therefore bears out the other data, and finds its explanation very simply in terms of the independent functioning of the rods and the cones.

V. INTENSITY DISCRIMINATION. a. *History and meaning.* Measurements of just perceptible intensity differences originally became significant because of their contribution to the problem of intensity recognition. Bouguer (1760), Steinheil (1837), Weber (1834), among others (for summary, see Hecht, 1924b) had shown that the sensory evaluation of intensity differences is relative and depends on the prevailing intensity. None of these data before 1860 could lay claim to any high precision. In fact, it was their very approximate nature which was of significance and which made them acceptable. If I and $I+\Delta I$ are two externally measured intensities which can be recognized as just perceptibly different in sensation, then the data showed that the just perceptible increment ΔI is not an absolute value which produces its sensory effect regardless of the prevailing intensity I, but rather that the two are related so that the ratio $\Delta I/I$ is roughly constant.

The error which came into the study of intensity discrimination,—and which has been perpetuated until this day as the Weber-Fechner Law,—arose when Fechner (1860), on the basis of poor measurements, supposed the fraction $\Delta I/I$ to be rigidly constant. Fechner's supposition of the constancy of $\Delta I/I$ for vision was immediately shown to be erroneous by Helmholtz (1866), even after only a few careful measurements, but it was Aubert (1865) who first demonstrated by extensive measurements with the human eye that $\Delta I/I$ is not constant, but varies in a specific way with I. His results have been corroborated by a variety of workers (see Hecht, 1924b, 1935a) during the last 75 years, not only for the eye but for the ear as well. For the eye $\Delta I/I$ decreases steadily from nearly 1 at low intensities to as little as 1/167 at high intensities.

The classic research on visual intensity discrimination has been the work of Koenig and Brodhun (1888, 1889) who found, as had Aubert and everyone since, that as I increased $\Delta I/I$ decreased. In addition, however, they found that with further increase in intensity $\Delta I/I$ again rose. This rise at high intensities has been generally accepted, and has formed an essential part of the theoretical explanations (Hertzsprung, 1905; Pütter, 1918; Hecht, 1924b, 1928; Houstoun, 1932) for intensity discrimination in vision.

Very recently the situation has changed fundamentally with respect

to this rise in the fraction $\Delta I/I$ at high intensities. First, the intensity discrimination of insects, as measured by Wolf (1933a, b) with the bee, and by Hecht and Wald (1934) (see also Wald and Hecht, 1933) with *Drosophila*, shows no rise in $\Delta I/I$ at high intensities; even when tested with intensities 10,000 times higher than the one at which its minimum $\Delta I/I$ becomes established, *Drosophila* showed not the slightest upturn in the value of $\Delta I/I$. Second, the validity of the upturn for the human eye itself has been seriously questioned by Guild (1932) who showed that the rise appears when the eye is insufficiently adapted to the prevailing intensity, and independently by Steinhardt (1936) who could eliminate it by surrounding the test-field with a large field of about the same brightness as the test-field, and by proper adaptation of the eye to the prevailing brightness. Indeed, Smith (1936) has just published measurements of intensity discrimination which show not a trace of rise at high intensities.

The upturn in $\Delta I/I$ has been so integral a part of the theories of intensity discrimination that its non-existence has rendered a new formulation necessary for the data (Hecht, 1934b; 1935). The new treatment describes all the available data including some measurements (Wright, 1935; Hecht, 1936; Smith, 1936) specifically designed to test it. Theory apart, however, the data themselves show a basic similarity for all animals studied, and furnish an interesting corroboration of the Duplicity Theory for the human eye.

b. *Insects, clam and man.* Intensity discrimination has been measured in insects, in *Mya*, and in man. The data for *Drosophila* are in figure 11. They are from the work of Hecht and Wald (1934) and were determined by the method of moving stripes used by Hecht and Wolf (1929) for measuring visual functions of animals with eyes. They represent the average measurements with 24 flies, and show that $\Delta I/I$ steadily decreases as I increases. The relation between the two is continuous, such as would be expected if just one photoreceptor system were concerned. The same is true for the data with the bee. Wolf's (1933a) first measurements with the bee are shown as solid circles in figure 12, while his later measurements (Wolf, 1933b), which are smoother, more numerous, and obviously more critical, are shown by clear circles. Though they were made with different sizes of stripes, the measurements are all essentially similar, and indicate most likely that only one set of photoreceptors is present.

Drosophila and the bee have organized eyes. The only other data of intensity discrimination available for invertebrates are for the clam

Mya, which has a diffuse sensitivity to light all over its siphon. The measurements with *Mya*, made twelve years ago (Hecht, 1924c), record the necessary fractional increase in illumination to which the animal responds with a specific reaction time, after having been adapted to a given intensity. Figure 13 shows the data for responses at five different reaction times. The measurements are not so smooth as can

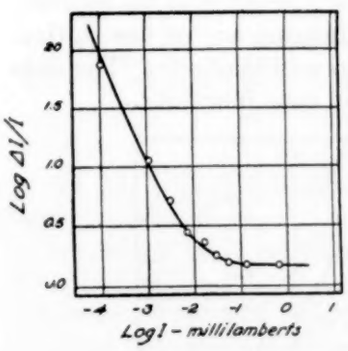

Fig. 11

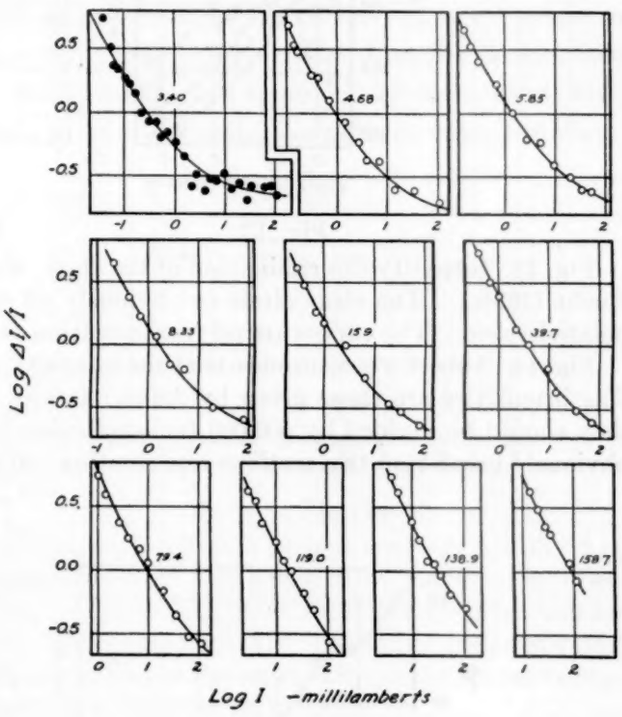

Fig. 12

Fig. 11. Intensity discrimination of *Drosophila* (Hecht and Wald, 1934). The points are the averages from 24 flies. The curve drawn through the data is from equation (10) derived in section IX.

Fig. 12. Intensity discrimination of the honey bee (Wolf, 1933a; b). The black circles are the data from the first paper; the plain circles from the second paper. The numbers attached to the curves are the visual acuities multiplied by 1000 and are inversely proportional to the size of the stripes used for the measurements. The same curve is drawn through all the data; it is from equation (11) derived later.

be wished, but they are consistent in showing that the relationship of $\Delta I/I$ to I is the same for all reaction times, and that it is a single function.

The remaining measurements of intensity discrimination are for the human eye, and when freshly examined two years ago (Hecht, 1934b) gave an unexpected demonstration of the separateness of rod and cone function. For their historical interest the earliest intensity discrimina-

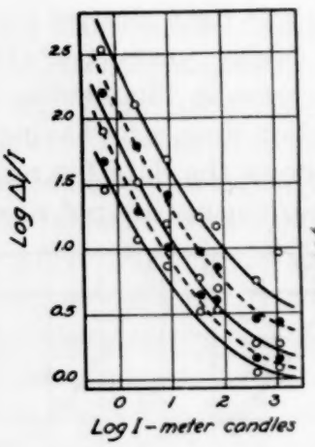

Fig. 13

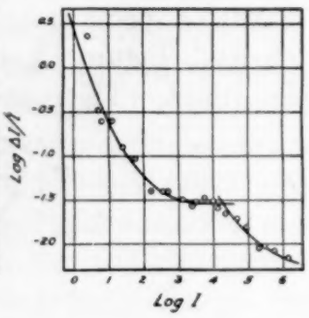

Fig. 14

Fig. 13. Intensity discrimination of the clam, *Mya*, from the measurements of Hecht (1924c). The clear circle so obviously off the topmost data is an extrapolated value. The curves are all from equation (11) derived later in this paper.

Fig. 14. Aubert's measurements of the intensity discrimination of his own eye. The intensities are those given by Aubert (1865). Judging by his description, they should be divided by 500,000 for conversion into millilamberts. The data obviously break into two sections representing rod and cone functions.

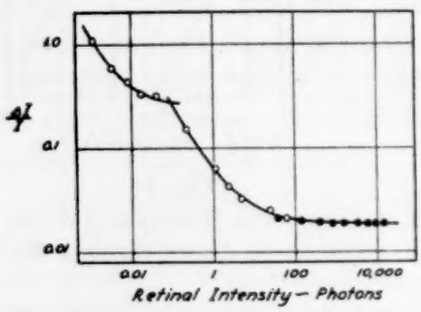

Fig. 15

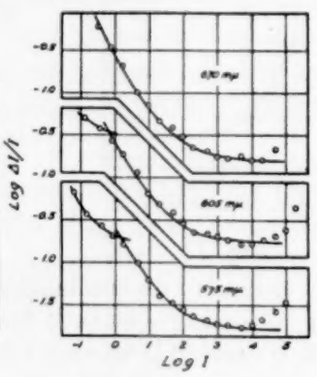

Fig. 16

Fig. 15. Intensity discrimination of the human eye. The measurements of Blanchard (1918) are the plain circles; those of Lowry (1931) are the black circles and have been raised 0.15 log unit along the ordinates to bring them into continuity with Blanchard's data. Note the natural breaking of the data into two sections indicative of rod and cone functions. The curve for the high intensity, cone data is from equation (11); the one for the rod data is actually from equation (10), but the other equation would do just as well. The equations are derived in section IX.

Fig. 16. Intensity discrimination for Koenig's eye with red, orange, and yellow spectral light (Koenig and Brodhun, 1888). The red data are continuous and show only cone function, whereas the orange and yellow show increasing amounts of rod function. As in figure 15, the curves are from equation (10) for the rod section and from (11) for the cones.

tion measurements (Aubert, 1865) are shown in figure 14. The points are single measurements and are fairly rough, but their significance is unequivocal. The data clearly range themselves into two parts as would be expected if they represent the behavior of two photoreceptor systems. That this phenomenon is not an isolated one is demonstrated in figure 15 which shows the measurements of Blanchard (1918) as open circles, and of Lowry (1931) as solid circles, both having been made in the same laboratory but thirteen years apart. The data break into two parts, of which the low intensity section is most likely concerned with rod function, while the high intensity section expresses cone function.

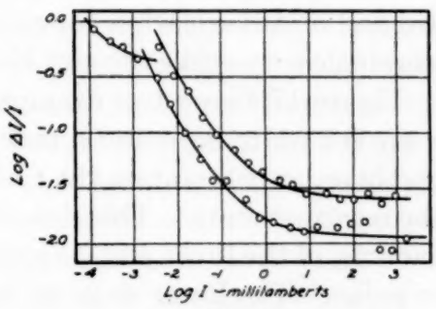

Fig. 17

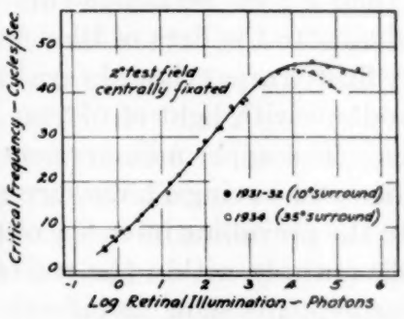

Fig. 18

Fig. 17. Intensity discrimination with different retinal areas, and with white light (Steinhardt, 1936). The upper data are with a field 56′ in diameter; the lower with a field 3°44′ in diameter. The upper data show only cone function and are described as usual by the curve from equation (11). The lower data show both rod and cone function; as in figures 15 and 16 the curve through the former is from equation (10), while through the latter it is from (11).

Fig. 18. Critical frequency and illumination. The data show a comparison for the same eye (S. H.) using central fixation and a 2° flickering field, between measurements made several years apart with a 10° surround (Hecht and Verrijp, 1933b) and with a 30° surround (Hecht and Smith, 1936).

c. *Color and position.* The two features which we have used in dark adaptation for distinguishing rod function and cone function are their differences in spectral sensibility and their differences in retinal location. These two characteristics are similarly useful in intensity discrimination. In terms of the visibility curves already shown in figure 1, we should expect that when the relation between $\Delta I/I$ and the intensity is measured with extreme red light, only the cone function should be apparent and the data should be continuous as they are with *Mya* and the insects. With orange light, however, we should expect the additional appearance of a small rod section, and this rod section should be larger as the spectrum goes toward the blue.

Koenig and Brodhun (1888) made measurements of $\Delta I/I$ with dif-

ferent spectral colors, and their data for red, orange, and yellow are reproduced in figure 16. No break is apparent for the data of 670 mμ, but a break is clearly shown in the 605 mμ and in the 575 mμ data. The low intensity section is small for the orange and large for the yellow. As already pointed out, the few points at high intensities showing a rise in $\Delta I/I$ must be disregarded because they were undoubtedly made under conditions which did not prevent the appearance of a rise.

The second method of demonstrating the separateness of rod and cone function is by measurements with differently located retinal areas, and has been used by Steinhardt (1936). For white light and test areas larger than 2°, his measurements without exception fall on a double curve similar to the data of Blanchard and of Aubert, while for smaller, foveally fixated areas they always form single curves like those of Koenig and Brodhun with light of 670 mμ. Figure 17 shows two examples of his data. The upper measurements are for white light and a test area 56′ in diameter having a large surround in order to maintain the eye as a whole to the prevailing intensity of the measurements. This size of test field falls entirely within the rod-free area of the fovea and, as a result, the measurements show no inflection point. The lower data in figure 17 were made with a field 3°44′ in diameter, also with a large surround, and show clearly the presence of a break indicating the existence of two separate functions,—rods at low intensities, and cones at high intensities.

Smith (1936) has recently published measurements of $\Delta I/I$ using fields of 20′ diameter, the two halves of which are separated by distances varying from 4′ to 30′, and in one case placed in separate eyes for binocular judgment. Judging by the field sizes, all his measurements fall in the rod-free area of the retina and, as expected, do not show any trace of discontinuity. The data thus corroborate Steinhardt's findings and support the general thesis of rod and cone identification.

VI. Intermittent stimulation. a. *Nature of problem.* The relation between visual function and the Duplicity Theory is nowhere so well illustrated as in the history and data of flicker. This is particularly apparent because this field of knowledge has undergone a rapid development and organization in the last few years.

The flickering sensation produced by regularly interrupted illumination disappears when the frequency of interruptions is made sufficiently high. The precise point at which flicker disappears is known as the critical fusion frequency, and may be determined with considerable accuracy. As a result, its value has been shown to depend on a variety of conditions.

The most basic factor which controls the critical frequency is the

intensity. Though the dependence of the critical frequency on illumination was recognized over 100 years ago by Plateau (1829) and is evident from the work of Emsmann (1854) and of Nichols (1884), it was only 45 years ago that Ferry (1892) formulated what has since become known as the Ferry-Porter Law, namely, that the critical frequency is proportional to the logarithm of the illumination intensity. Ferry's published measurements support this formulation only in the most general way, but the later data of Porter (1902) are adequate for its statement. Porter's work was corroborated by Kennelly and Whiting (1907), by Ives (1912), and by Luckiesh (1914).

Porter found that when the critical fusion frequency—as cycles of light and dark per second—is plotted against the logarithm of the intensity, the data fall on two straight lines instead of one. The two lines intersect at an illumination of about 0.25 meter candle, and the slope of the lower is 1.56 while that of the upper is 12.4. These findings were confirmed by Ives (1912a), whose data for different parts of the spectrum also show a dual logarithmic relation. However, the slope of the straight lines and their point of intersection seem to vary with the wavelength of the light, the upper and lower limbs of the relationship varying in different ways. In addition, Ives found the extraordinary fact that for blue light the lower line becomes horizontal.

These peculiarities are difficult to reconcile with the obvious interpretation of Porter's data in terms of the Duplicity Theory, and this difficulty has been emphasized by Allen (1919, 1926) who has drawn through his measurements about five short, straight lines of different slope instead of the usual two. Judged by their experimental error, the data presented by Allen do not justify this treatment; the points most probably lie on a continuously curving line. The work of Lythgoe and Tansley (1929) distinctly gives no support to Allen's multiplicity of straight lines.

Lythgoe and Tansley's measurements confirm the logarithmic relation of intensity to fusion frequency, but Lythgoe and Tansley attach no importance to its strict formulation as done by Ferry, by Porter, and by Ives, and consider that their data agree only under certain conditions with the linear relation of critical frequency to log I. The same may be said about the measurements of Granit and Harper (1930), who found that for a range of about 1 to 1000 in intensity the critical frequency is very nearly directly proportional to the logarithm of the intensity. For higher intensities the relationship does not hold, and the curve of frequency against log I tends to become horizontal, as already found by Grünbaum (1898).

One striking thing appears in the work of Lythgoe and Tansley though they do not recognize its significance. Ives had found that for blue light the lower limb of his data is horizontal, and in this he had been confirmed by Allen. This seemed a special property of blue light. However, Lythgoe and Tansley have recorded that when measurements are made with a retinal area 10° from the center of the eye the lower portion of the data tends to be horizontal even for white light.

As a result of these researches, it might seem that flicker data make no sense in terms of the Duplicity Theory. In fact, it was precisely this confusion which prompted me originally (Hecht and Verrijp, 1933b) to study flicker; this, and the fact that none of the measurements existing at the time covered a range of intensities sufficient to define the relation between critical frequency and intensity over the functional range of the eye. Since then, we have measured this relation for different portions of the retina, for different sizes of field and for different colors, for as large a range of illuminations as possible, and under such conditions of fixation and surrounding illumination as to render the data reproducible and definitive. As a result the confusion has disappeared, and the conflicting data have become integrated into a scheme which is consistent with the visual knowledge already presented and understood.

b. *Central and peripheral measurements.* We may again use the type of thinking and experimentation which the previous sections have shown to be valid in differentiating between rods and cones. If the separation of rod and cone function first suggested by Porter's data is correct, it should be possible to isolate them in the usual way,—first, by studying different retinal areas, and second, by means of spectral illumination. Judging by the distribution of cones and rods, the relation between fusion frequency and intensity, as measured with central areas smaller than 2° in diameter, should be a continuous function representing cones, whereas with larger areas or with similar small areas outside the fovea, the relation should show a duplex character illustrative of the predominant working of rods at low intensities, and of cones at high intensities.

The measurements of Hecht and Verrijp (1933b) with a small field located centrally and peripherally show this to be correct. Figure 18 shows two sets of measurements on my own eye, made several years apart (Hecht and Verrijp, 1933b; Hecht and Smith, 1935; 1936), with a 2° field situated in the fovea. The data demonstrate that for the fovea there is one continuous relation between critical frequency and the logarithm of the intensity. The relationship is distinctly sigmoid, the S-shape being rather drawn out. In the middle range of intensities, the

data lie with reasonable precision on a straight line, and thus confirm Porter, Ives and the other workers, even to the extent of having a slope of the same magnitude as found by them.

Below the middle range the data form a gentle curve which stops fairly abruptly when with central fixation the field appears uniform even when the test area is extinguished. At the highest intensities the relation between critical frequency and log I rapidly ceases to be linear. The curve flattens out, and as with intensity discrimination, remains flat provided the adaptation and surround are adequate.

Using the same sized field (2° in diameter with a non-flickering 10° surround), we measured the relation between critical frequency and in-

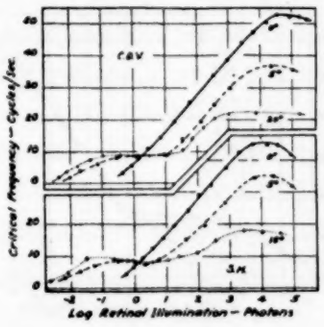

Fig. 19

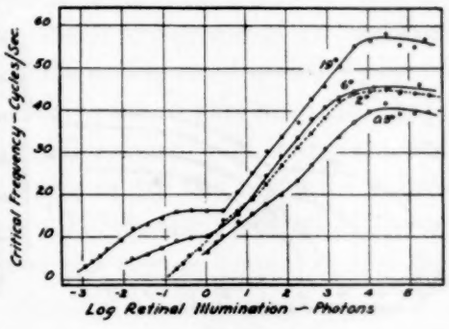

Fig. 20

Fig. 19. Relation between critical frequency and log I for white light with a 2° field in four retinal locations: at the fovea, and at 5°, 15° and 20° above the fovea. The data are from Hecht and Verrijp (1933b). Due to an error in the original paper, the intensities have had to be multiplied by 40 to convert them correctly into those here given.

Fig. 20. Influence of the area of test field on the relation between critical frequency and log I (Hecht and Smith, 1936).

tensity at 5°, 15°, and 20° from the center. The results are shown in figure 19, and are strikingly different from the central data in that they clearly fall into two parts. The first is at low intensities, where the critical frequency first rises with log I and then reaches a maximum which is maintained approximately constant for about 1.5 logarithmic units. The total intensity range covered by this rise and plateau is about 3.5 logarithmic units. The second part also begins with a rise in critical frequency as log I increases, and also terminates when the critical frequency reaches a maximum. The intensity range covered by the second part is about 4 logarithmic units. The same results obtain in whatever peripheral direction of the eye the measurements are made.

Since the central, 2° field falls within the relatively rod-free area of the retina, the continuous nature of the data mark them as a function of the cones alone. The double nature of the peripheral measurements very likely represent rod function for the low intensity section and cone function for the high intensity section. This is borne out by the increasing separation of the two sections as measurements are made farther and farther from the center: the cone section shifts to higher intensities and the rod section to lower intensities, as would be expected from the increasing ratio of rods to cones in these regions.

We have very recently measured the relation between critical fusion frequency and intensity for four centrally located areas 0.3°, 2°, 6°, and 19° in diameter, and our measurements (Hecht and Smith, 1935; 1936) confirm these conclusions. The data for E.L.S. are in figure 20 and, as expected, those for 6° and 19° break sharply into a rod and cone section, while the 2° and 0.3° fields fail to show the rod section. The slight bend in the latter data need not concern us here; it is certain that the bend is not due to a slight admixture of rods.

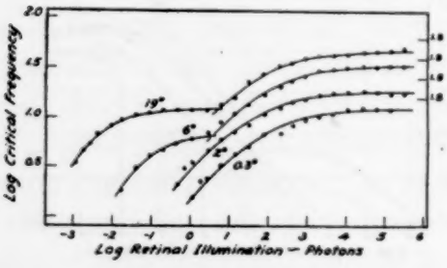

Fig. 21

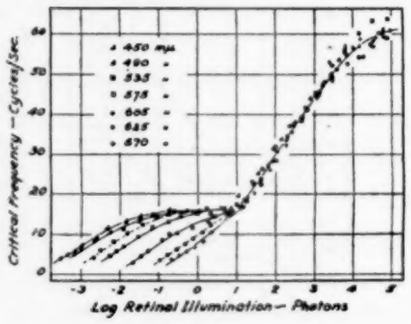

Fig. 22

Fig. 21. Area and the flicker relation. The log I axis is the same for all the data. The numbers on the log frequency axis to the left apply to the uppermost data only; the other data have been moved down in steps of 0.2 log unit in order to space them, and their precise position is given on the right. The curves are from equation (14) in which for the cone portions $m = 2$, $n = 2$; and for the rod portions $m = 2$, $n = 1$. Cf. figure 29 shown later.

Fig. 22. Critical frequency and log I for different parts of the spectrum for the eye of S. H. (Hecht and Shlaer, 1936).

The data for the 6° and 2° fields are of pointed interest in the problem of flicker and area. Except for the absence of the rod piece in the smaller field, the two sets of data are almost identical. Under the circumstances of possessing the same surround, a ninefold increase in area of the test field hardly changes the relation of critical frequency to in-

tensity so far as cone function is concerned (*cf.* Granit and Harper, 1930).

Figure 21 presents the data of S.H. as the logarithm of the critical frequency against the logarithm of the intensity. This type of plot shows more strikingly the phenomena already described. In spite of the irregularity in the 0.3° data, a single curve describes the measurements fairly well. The same single curve is even more expressive of the 2° data, and it is also drawn through the cone portions of the 19° and 6° data.

The rod sections of 19° and 6° require a slightly different curve which is the same for the two fields. It is worth emphasizing that the rod sections of the two large fields have the same curve drawn through them. While this is not clearly seen in an ordinary graph of critical fusion frequency against log I, it becomes plain in the log I – log f plot of figure 21. This is because on a log I – log f plot of this kind the shape of the curve relating critical frequency and intensity is invariant and uninfluenced either by the intensity units or by the absolute values of the critical frequency, since these merely shift the position of the curve on the two axes. The same is true of the curves shown in the section on intensity discrimination. The identity of the curves for 19° and 6° shows that the difference between them is not basic, but merely represents a change in one of the constants in the equation which describes them.

Exactly the same is true for any systematic differences which the cone data show. Fundamentally the systems in the rods and cones which determine the relation between critical frequency and intensity remain the same regardless of area. Only the parameters are altered by changing the area. This has finally been recognized by some of the investigators who are interested in studying the influence of various factors on visual functions. For example, Smith (1936) who was essentially concerned with synaptic and other nervous influences on intensity discrimination has found that the fundamental intensity discrimination relation remains unaltered, and that the best way to describe these various nervous influences is to record their effects on the parameters of the basic equations (Hecht, 1934a, 1935) for intensity discrimination. The same thing is clearly possible with flicker, and should be done before the determination of fusion frequency is rushed into medicine for use as a clinical method (Granit, 1936).

c. *Spectral data.* In order to confirm the identification of the two sections shown by the measurements which include the periphery, we

have used different parts of the spectrum to study the relation of critical frequency to intensity. It is worth looking again at figure 1 in order to see what may be expected. Spectral energy can produce no visual effect until it reaches the relative intensity indicated by the rod curve. Above that, rod function dominates until the cone threshold is reached. The intensity distance over which the rods dominate in visual function changes throughout the spectrum: between 670 and 630 mμ it is small and alters only slowly; beginning at about 600 mμ and going toward the blue the distance becomes rapidly larger, while below 500 mμ it remains practically constant.

Preliminary investigation (Hecht and Verrijp, 1933a) showed these expectations to be correct. We therefore measured in detail (Hecht and Shlaer, 1935; 1936) the relation between intensity and critical frequency for different parts of the spectrum with a circular test field 19° in diameter, surrounded by a non-flickering area 35° in diameter.

The information conveyed by the measurements can best be understood from their graphic representation. As figure 22 shows, the data break sharply into two sections. The high intensity portions, identified with cone function, fall together for all the colors. The low intensity sections, identified with rod function, are spread out much as expected and extend to lower and lower intensities with decreasing wavelength.

The separation between rod and cone sections for white light shown in figure 20 almost coincides with that for 535 mμ (yellowish-green). The separation for white light is dependent in part on the integrated spectral sensibilities for rods and cones, and in part on their absolute thresholds. Wolf and Zerrahn-Wolf (1936) have just reported critical frequency measurements for the sunfish *Lepomis* used by Grundfest (1932b) for his spectral visibility studies. Just as Grundfest found two visibility curves, so Wolf and Zerrahn-Wolf find two limbs to the flicker data indicating rod and cone function. The intensity separation of the two limbs is more than 4 log units, the main rod section being almost impossible to measure because it lies below the threshold of human vision.

Figure 22 resolves the mystery of Ives' old measurements showing that the low intensity portions of critical frequency data which he found for different parts of the spectrum may be represented by straight lines which differ in slope, the red being steepest and the violet being practically horizontal. It is apparent in figure 22 that for short stretches near the rod-cone transition, straight lines can be drawn through the rod data, showing different slopes for the different wavelengths.

The real phenomenon, however, is something quite different. It is that the separation of rod and cone sections as a whole increases as the wavelength decreases. This is shown strikingly by figure 23, in which the data of S.S. are plotted as the logarithm of the critical frequency against the logarithm of the intensity. The data for 670 mμ fall on a single, continuous curve, whereas the data for all other parts of the spectrum are best described by two separate curves. The transition between the two portions is quite sharp for all but the blue and violet

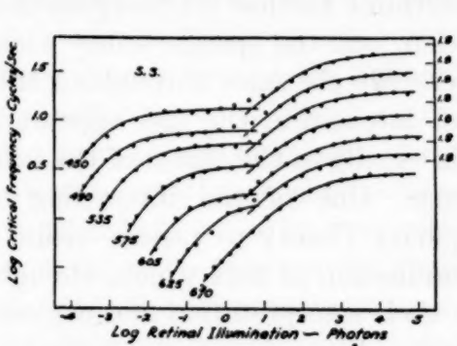

Fig. 23

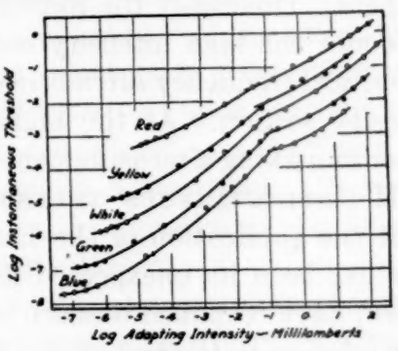

Fig. 24

Fig. 23. Critical frequency and intensity for different spectral regions for the eye of S. S. (Hecht and Shlaer, 1936) plotted as log frequency against log intensity. The numbers on the ordinates to the left apply to the topmost data; for convenience, the others have been moved down in steps of 0.2 log unit, and their exact positions are indicated to the right. The curves are from equation (14) in which for the cone portions $m = n = 2$, and for the rod portions $m = 2$, $n = 1$.

Fig. 24. Relation between adaptation intensity and instantaneous threshold for different colors (Blanchard, 1918). The adapting intensity axis is the same for all the data. The numbers on instantaneous threshold axis apply only to the data for white light. To avoid confusion the yellow data have been displaced upward 0.5 log unit; and the red 1 log unit; the green downward 0.5 log unit; and the blue 1 log unit. Actually the high intensity portions of all the colors practically coincide, whereas the low intensity sections drop more and more from the red down.

data. The high-intensity, cone curve is in the same position for all colors, and the only effect of changing the spectral composition of the light is to shift the position of the low-intensity, rod curve along the intensity axis without in the least changing its form.

The identification of rod and cone function is borne out by subjective observation. At low intensities and below the critical fusion frequency the flicker is distinctly located in the peripheral portion of this 19° field so that the field resembles a flickering doughnut, and the last appearance of flicker is always in the periphery. With increasing inten-

sity the first sign of approaching cone function is the appearance of color in the field, which becomes identifiable with certainty about 0.5 log unit below the actual inflection point of the measurements.

At the intensities around the transition, two separate loci of flicker are very often apparent near the critical frequency: one in the periphery, and the other in the center. At intensities higher than the transition intensity but near it, flicker usually persists longest in the center, but beyond these intensities the last trace of flicker may be in any part of the field. Obviously the rods determine the low intensity section and the cones the high intensity section, but the specific cones which set the critical frequency are not necessarily the same throughout the high intensity section. At the highest intensities, the last appearance of flicker in making a measurement is usually in the center of the field.

VII. Instantaneous threshold. One of the interesting things about the application of the Duplicity Theory to various visual functions has been its unexpected illumination of data which, though well known, have remained obscure in their interpretation and neglected in their relation to the rest of the field. A case in point is Aubert's data of intensity discrimination which have been known for about 75 years and have been frequently quoted, but were never recognized as demonstrating the Duplicity Theory until now. I myself had studied these data many times, but never saw their separation into rod and cone sections until in 1934 when I plotted them as log $\Delta I/I$ against log I, as shown in figure 14. Under these circumstances the division almost leaps out from the data by itself. The same thing happened with Blanchard's measurements shown in figure 15, and with those of Koenig and Brodhun in figure 16.

An even more curious situation concerns the data of Blanchard (1918) on instantaneous thresholds. Blanchard's measurements were of the following kind. The eye is adapted to a given intensity by looking for some time at a large, suitably illuminated area. At a specific moment this adapting light is cut off with a shutter, and at the same time the minimum intensity is determined at which a centrally-fixated, 5° area becomes visible instantaneously. This naturally requires several trials, each preceded by proper adaptation, until the precise threshold intensity is found which renders the central area just visible immediately after the adapting light is shut off.

Blanchard made these measurements over the whole range of visually effective intensities, using white light as well as the red, yellow, green, and blue portions of the spectrum isolated by roughly monochromatic

filters. He found that the instantaneous threshold intensity increases with the adapting intensity, and that on a double logarithmic plot "the curve for white light is seen to be practically a straight line with the exception of the extreme intensities, and may be represented over this region by the" appropriate equation for a straight line. He says further that "the curves for the different colors are very similar to that for white, the instantaneous thresholds being nearly equal for moderate brightnesses and diverging most for the lowest brightness," and correctly attributes the latter fact to the Purkinje phenomenon.

Blanchard adds the pertinent observation that "all the color curves except red show a decided dip, indicating a depression of sensibility, in a region roughly between 0.01 and 1 milliambert, a region corresponding to about the average range of interior brightnesses at night," but fails completely to appreciate the meaning of his observation. I have replotted his data in figure 24, from which the significance of his "dip" is at once apparent in terms of the Duplicity Theory.

With red light the measurements show almost a continuous function. When plotted by themselves the red data may easily be fitted with one line, as were those of intensity discrimination in figure 16 and of flicker in figure 23. In fact I did precisely this even while making the first draft of figure 24 for the present paper. It is only when the data for the other colors are added that the double nature of the function becomes apparent, because the inflection point in the red data, though gentle, corresponds so obviously with the point of striking change in slope shown by the yellow, white, green, and blue data. Judged by the transmission data published by Blanchard, his red light included the spectrum beginning at 600 mμ; it would therefore be expected to stimulate the rods as well as the cones in this 5° centrally-fixated area.

Figure 24 shows that the high intensity portions for all the colors and for white are much the same, indicating their common origin in cone function. Except for the red, the low intensity sections drop sharply away also in a form which is approximately the same for all the measurements. Note particularly that the low intensity rod section is largest for blue light and becomes smaller through the spectrum to the red, as is to be expected from the sensibility curves in figure 1. Considering all that has already been said about similar things in dark adaptation, intensity discrimination, and flicker, it is hardly necessary to labor the point of these data.

VIII. Visual acuity. a. *Normal eye.* The last function to be considered in relation to the Duplicity Theory is the resolving power of the

eye and the influence of illumination intensity on it. A measure of the capacity of the eye for resolving the details of its environment is visual acuity. This is defined as the reciprocal of the angular distance which must separate two contours in order that they may be recognized as discrete, the unit of separation being a minute of arc. The relation between visual acuity and illumination was first investigated in 1754 by the astronomer Tobias Mayer. He believed that he had found that visual acuity increases as the sixth root of the intensity of illumination.

Since Mayer, many investigators have tried to find the exact nature of the relationship between visual acuity and illumination. Uhthoff (1886), who reviewed the data 50 years ago, records about twenty contributions to this particular phase of vision. Most of these data cover only a very small range of illuminations, and therefore merely confirm daily experience. Nevertheless, even a small range enabled Posch (1876) to conclude that visual acuity varies very nearly as the logarithm of the illumination intensity. This relation is borne out by the more comprehensive data of Klein (1873) and Cohn (1883), though neither of these authors drew such a conclusion.

Uhthoff (1886; 1890) himself investigated the matter further by using white and colored lights covering a great range of illuminations. From his data it is apparent that in a general way visual acuity varies in proportion to log I; the relation between the two is not strictly rectilinear, but sigmoid. His data are excellent, and were confirmed and extended in great detail by Koenig (1897) whose measurements have since become classic.

Since Uhthoff's and Koenig's experiments the number of people who have measured this relationship is not large. Up to 1912 they are recorded in an excellent memoir by Löhner (1912); an earlier review is by Zoth (1905). After that, none of the contributions (Rice, 1912; Roelofs and Zeeman, 1919; Ferree and Rand, 1923; Lythgoe, 1932; Wilcox, 1932) have covered the range of illumination necessary for a complete statement of this function of the eye. They all indicate, however, that the relation between visual acuity and illumination is of the general form found by Koenig.

In 1926 I made an interpretation of these data (Hecht, 1926, 1928) in terms of certain photochemical and statistical notions which accounted for the precise form of the relationship. As a result of these ideas, it became necessary to know the influence of intensity on visual acuity for animals other than man. Such studies were soon forthcoming (Hecht and Wolf, 1929; Hecht and Wald, 1934; Clark, 1935),

and showed that for all animals the general form of the relationship is much the same as for man.

From the point of view of rod-cone function, it is not necessary to know the precise form which the data for the human eye assume. The significance of Koenig's data in this connection is clear enough, and was to a certain extent recognized by him as well as by von Kries (1929). However, their full value for the separation of rod and cone function becomes apparent only when they are plotted in a double logarithmic grid as in figure 25, since in this way the lower values of visual acuity receive their share of importance, and the experimental error as a percentage is of the same magnitude throughout.

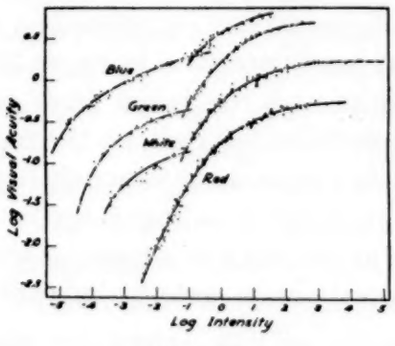

Fig. 25

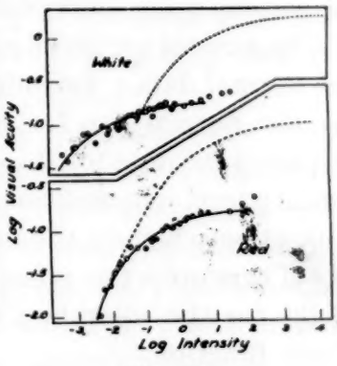

Fig. 26

Fig. 25. Visual acuity and illumination for different colors (Koenig, 1897). The intensity axis is the same for all the colors. The numbers on the visual acuity axis apply only to the data for white light. For convenience in showing the data, the red measurements have been displaced downward 0.5 log unit; the green, 0.5 log unit upward; the blue, 1 log unit upward. The separation of the data into two sections needs no emphasis.

Fig. 26. Visual acuity and illumination with red and white light for a completely colorblind person (Koenig, 1897). The white curve and the red curve from the normal data of figure 25 have been drawn in for reference.

From figure 25 it is apparent that with red light there is only one continuous relationship between visual acuity and intensity, while for white, green, and blue there are two relationships, one at high intensities and the other at low intensities. Koenig had considerable difficulty with blue light and could not complete the measurements at high brightness and high visual acuity. The high intensity limb for all the colors is the same as for red and must therefore represent cone function; by the same token the similarity of the low intensity portion for blue, green, and white, and its complete absence in the red, mark it as the expression of rod function. Note particularly the position on the intensity axis

and the relative size of the rod portions for blue, green, and white. The blue section is largest, the green next, the white next, and the red is non-existent, due to the movement of the rod section along the intensity axis, as in figure 5 for dark adaptation and in figure 23 for flicker.

b. *Complete colorblindness.* Koenig recognized the implications which his visual acuity measurements have for rod and cone behavior because his work was done almost immediately after von Kries had reintroduced the idea of duplex function into visual physiology. Koenig realized that in a completely colorblind individual the upper part of the data should disappear and leave only the lower limb plus any extensions of it. He accordingly investigated the visual acuity of a completely colorblind person, using white and red lights.

The data he secured are given in figure 26 and are plotted on the same grid as the normal data. Included for comparison in figure 26 are the curves for the normal eye for white and red taken from figure 25. With white light the data for the colorblind fall right on the rod portion of the normal curve. In addition they extend continuously beyond the point of inflection which in the normal curve indicates the entrance of the cones. There are a few points at the highest intensities with white and red light for the colorblind which may perhaps indicate a rudimentary cone function.

The data for red light are particularly revealing. They have much the same shape as the rod portions of the blue, green, and white data for the normal eye. But they suffer a shift along the intensity axis as do the rod data of the red flicker measurements in figure 22, and end up just to the right of the normal red data, being coincident with them at the lowest intensities. In figure 26 the position of the colorblind red data under and to the right of the normal data explains why the normal red curve is continuous and without a break. It is not that the rods do not function in red light; it is that in red light the cones have about the same intensity thresholds as the rods (*cf.* fig. 1); but since the cones are closer together in the fovea than are the rods in the periphery, they can resolve smaller distances and therefore dominate the measurements by recording the correspondingly higher visual acuities.

The dominating characteristic which differentiates rod and cone function in visual acuity is obviously purely anatomical. For other visual capacities the dominating differentiation may be quite different and may depend on the properties of the photoreceptor mechanism. In dark adaptation it is very likely the speed of the regenerating chemical process which serves to separate rods from cones in the measurements. For

intensity discrimination and flicker it may be the difference in the just effective photochemical change in the receptor cell. No matter what the basis for the particular difference in rod and cone function happens to be, it gives expression to the spectral sensibility curves and becomes revealed in the measurements.

IX. THE NATURE OF THE PHOTORECEPTOR PROCESS. a. *The stationary state.* The first purpose of this paper has been to show that the various visual functions which have been measured with precision fall quite simply into the design of the Duplicity Theory. This objective has now been accomplished, and has involved little more than the presentation of the data themselves. The second purpose is to go somewhat deeper into the quantitative meaning of the measurements, and to consider their relation to the processes which probably occur in the rods and cones when they are activated by light.

The most general ideas that one can have about the photoreceptor process involve three things. *a.* There has to be a sensitive substance which absorbs light and is changed by it into one or more active products. *b.* It is necessary to maintain a supply of this photosensitive material; otherwise it would be used up and the process would come to an end. These two processes may be called the primary light and primary dark reactions. *c.* The active photoproducts of the primary light reaction must do something of which the end result is an impulse from the receptor cell. This is the secondary dark reaction.

These three requirements are the minimum essentials. The photoreceptor process is certainly more elaborate. Moreover, vision and light sensitivity involve not only the receptor process in the sensory elements but the nerve impulses generated by the stimulated elements and by neighboring elements, as well as all sorts of central nervous changes of which we know little or nothing. Since these are all concerned with vision, they surely influence its characteristics to some extent. However, no matter what determines the nature of vision, the ultimate place of origin of the impulses passing up the optic tracts is in the action of light on the sensory elements. Therefore the various properties of vision and photoreception must owe some of their quantitative form to the characteristics of the reactions which take place between light and the sensitive elements. It is obviously of first rate biological interest to discover how close this correlation is.

Consider the properties of the simplest photochemical system which can be suggested as corresponding to the primary light and dark reactions. Let there be a photosensitive substance whose total initial con-

centration is a; let light of intensity I shine on it; let the concentration of photoproducts at the moment t be x; and let it be assumed that some of these products reunite by themselves or with the help of additional substances to form again the sensitive material. The velocity of the process as a whole will then be

$$(dx/dt)_I = k_1 I\ (a - x)^m - k_2 x^n \tag{4}$$

where m and n represent the order of the primary photochemical reaction and of the primary dark regenerating reaction respectively; and k_1 and k_2 are their velocity constants, k_1 including the absorption coefficient. The intensity enters as the first power because this is the only assumption compatible with Talbot's law (Arnold and Winsor, 1934), as well as with ordinary absorption ideas as shown in equation (3) in the third section of this paper.

On continuous illumination corresponding to the adaptation of the eye to a constant intensity of light, such a photochemical system reaches a stationary state in which the opposing reaction rates become equal; the concentrations of sensitive material and photoproducts become constant; and equation (4) becomes equal to zero. This gives

$$KI = x^n/(a - x)^m \tag{5}$$

where $K = k_1/k_2$ for convenience.

Equation (5) describes the photostationary state set up by light in terms of the simplest chemical ideas about the primary light and dark reactions. Examination of the data reveals that intensity discrimination, visual acuity, and flicker all depend on light intensity as if they were determined by the conditions at the stationary state described by equation (5). In fact, so close is the relation between the equation and the data, that it is possible to determine from the data the precise values of m and n in the equation.

b. *Intensity discrimination.* When the eye is exposed to light of intensity I, the combined rate of the primary light and dark reactions in the photoreceptor process is that given by equation (4). At complete light adaptation to this intensity the velocity becomes zero, and the equation becomes.

$$I = k_2 x^n/k_1\ (a - x)^m. \tag{6}$$

If the system is now exposed to intensity $I+\Delta I$, which is the intensity just perceptibly brighter than I, the initial velocity will be

$$(dx/dt)_{I+\Delta I} = k_1\ (I+\Delta I)\ (a - x)^m - k_2 x^n, \tag{7}$$

no changes in concentration having yet taken place. Subtracting equation (4) from (7), we get

$$(dx/dt)_{\Delta I} = k_1 \Delta I\ (a - x)^m. \tag{8}$$

Assume that the just perceptible increment in intensity ΔI is recognized when $(dx/dt)_{\Delta I}$ is constant and equal to c'. This probably means that in a short time Δt, a constant increment of sensitive material Δx, must be decomposed by the addition of ΔI; this small increment Δx may show itself as a given increment in the frequency of impulses leaving the receptor cell to the associated nerve fiber (*cf.* Adrian and Matthews, 1927; Hartline, 1934). Equation (8) then gives $\Delta I = c'/k_1\ (a - x)^m$. Dividing this value of ΔI by the value of I from equation (6) we get

$$\Delta I/I = c'/k_2 x^n \tag{9}$$

as a description of $\Delta I/I$ in terms of the general photoreceptor system. In other words intensity descrimination varies inversely as some power of the concentration of photoproducts present at the stationary state.

In order to compare this general derivation with specific data which give $\Delta I/I$ against I, it is necessary to replace x by I derived from the stationary state equations (5) and (6), and this requires specific values for m and n. When m and $n = 1$, that is, when both the primary light and dark reactions are monomolecular, equation (9) becomes

$$\Delta I/I = c\ (1 + 1/KI) \tag{10}$$

where $K = k_1/k_2$, and $c = c'/ak_2$. When $m = n = 2$, that is when both reactions are bimolecular, equation (9) becomes

$$\Delta I/I = c\ (1 + [1/KI]^{\frac{1}{2}})^2 \tag{11}$$

where $c = c'/a^2k_2$. Equations (10) and (11) are shown graphically in figure 27; with them are the two curves expressing the situations when $m = 1, n = 2$, and $m = 2, n = 1$.

So far as the data of intensity discrimination are concerned only the symmetrical cases shown in equations (10) and (11) need be considered, because between them they describe all the measurements. Equation (10) is the curve which is drawn through the *Drosophila* data in figure 11. Equation (11) forms the curve for all the bee data in figure 12, for the data for *Mya* in figure 13, and for all the cone data of the human eye in figures 14, 15, 16 and 17. All these data are quite critical, and there is no difficulty in deciding which curves fit which set of measure-

ments. Smith's (1936) measurements of foveal intensity discrimination, made to test these ideas critically and perhaps antagonistically, have been shown by him to correspond to equation (11) with extraordinary precision.

For the rods of the human eye it is not possible to decide which equation applies because the data are too few; all four equations are equally adequate.

c. *Visual acuity.* Measurements of visual acuity depend on the recognition of a space between two contours. Because of the discrete

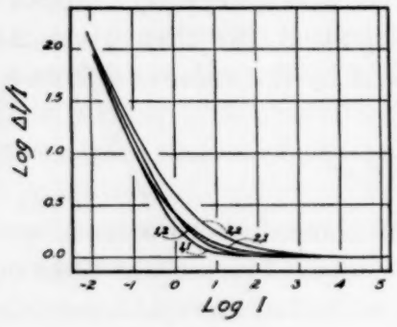

Fig. 27

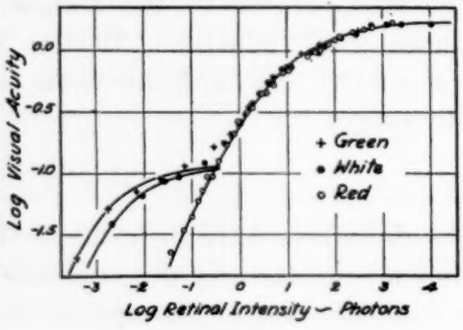

Fig. 28

Fig. 27. The relation between $\Delta I/I$ and I in terms of the theoretical derivations. Drawn on a logarithmic grid, the curves have a form which is independent of any constants in the equations, but which is determined by the values of m and n representing the order of the primary photochemical and dark reactions in the photosensory system. The numbers attached to the curves show the value of m and n in order.

Fig. 28. Relation between visual acuity and retinal brightness. The original data are from Koenig (1897) and are shown in figure 25. For convenience, they have been averaged in groups of five. The intensities have then been converted to millilamberts, and multiplied by the corresponding average pupil area, using the data of Reeves (1918), by the pupil efficiency factor from the data of Stiles and Crawford (1933), and by $10/\pi$ to convert them into effective photons. The curve through the cone portion of the data is the 2,2 curve (upside down) from figure 27, while those through the low intensity rod portions are the 2,1 curve (upside down).

nature of the retina, this recognition requires the presence in the space of one element, or of a given number of elements, which are stimulated to a different extent than are the nearby elements which are covered by the two contours. Visual acuity varies with intensity; therefore this just recognizable space varies in size with intensity, from which it follows that the constant number of elements required for the recognition of this space will occupy a variable area depending on the intensity. This is a conceivable situation, and has indeed been described in conformity with the data in terms of certain ideas about the statistical

variation in threshold of the elements in the retinal population (Hecht, 1926, 1928). This statistical treatment of visual acuity remains today much as when it was proposed; it describes the data quantitatively, and no other quantitative explanation has been proposed. I have discussed this matter elsewhere (Hecht and Wald, 1933) and need not elaborate it here.

The whole problem of visual acuity and intensity may also be looked at from a different point of view, and I present it here because of its simplicity and suggestiveness. Two lines are resolved as a doublet when the space between them appears of a different brightness than the lines themselves. An object is seen as a C or an O depending on whether the circular image formed on the retina contains a break whose brightness is recognizably greater than the rest of the circle. In other words, visual acuity may be intimately related to intensity discrimination, a suggestion first made in a rather general way by Helmholtz (1896) and later by Hartridge (1918) who studied it in detail for the special case of maximum retinal resolution.

Intensity discrimination varies with the prevailing intensity and so does visual acuity. If the two functions are in some way related, they might bear the same relation to intensity. Intensity discrimination is measured as the fraction $\Delta I/I$, and this fraction decreases as the intensity increases. Visual acuity should therefore vary inversely as $\Delta I/I$, which means in terms of equation (9) that it should vary directly as some power of the concentration of photoproducts at the stationary state. Putting visual acuity $V = bI/\Delta I$, and substituting in equation (9) we get

$$V = cx^n \tag{12}$$

where $c = bk_2/c'$ as a lumped constant.

The further solution of this equation in terms of specific values of m and n is exactly the same as for intensity discrimination. In fact, the final curves plotted on a double logarithmic grid are identical with those in figure 27, but reversed in the sign of the ordinates. Instead of going down, the curves go up in mirror image but have exactly the same forms.

Do visual acuity data fit these curves and equations? It must be recalled that the equations apply to the retina, and that intensities at the retina correspond to outside intensities only when the pupil area is constant. The measurements of intensity discrimination were made with constant pupil. Koenig's measurements of visual acuity were unfortunately made with the natural pupil, as were most of the other

measurements discussed in section VII. I once (Hecht, 1928) corrected Koenig's measurements in terms of the pupil data of Reeves (1918). Since then Stiles and Crawford (1933) have shown that the pupil is not efficient in transmitting light in proportion to its area, and an additional efficiency factor must be added.

The original measurements of figure 25 have been averaged in groups of 5, corrected for pupil area according to the average of Reeves, and for pupil efficiency according to Stiles and Crawford, and are now all put together in figure 28. Through their common, high intensity cone portion the 2,2 curve of figure 27 has been drawn (upside down, of course). It is the only one of the four curves which approximates the data. The fit is not so good as one would like; and the reason for it, as we know from measurements now being made in our laboratory by Dr. Simon Shlaer, is that Koenig's cone measurements were not confined to a single retinal location: the lower cone portion is parafoveal while the upper is foveal. Shlaer finds that when the measurements are deliberately confined to the fovea, the correspondence between the data and the 2,2 equation is nearly perfect.

The rod portions of the white and green data definitely do not fit either the 1,1 or the 1,2 curves. They do fit the 2,2 and the 2,1; the data are not sufficiently critical for a choice. The curve drawn through them is actually the 2,1 curve, for reasons which will become apparent in a moment.

d. *Flicker.* Intermittent stimulation by light is interesting because it makes an extra demand on the very simple receptor system suggested at the beginning of this section. Intensity discrimination and visual acuity have relied solely on the stationary state of the primary light and dark reactions. Flicker involves in addition the secondary dark reaction which follows them.

Under the influence of light, both primary reactions proceed, and the velocity of their combined reaction is given by equation (4) derived previously. In the absence of light, the primary dark reaction goes alone, and the equation

$$-(dx/dt)_{\text{dark}} = k_2 x^n \tag{13}$$

gives the rate at which it forms the photosensitive material. In intermittent illumination these two reactions alternate rapidly, and form a steady state in which what has been decomposed during the light period is regenerated during the dark period. Since the light and dark periods in all the measurements given in section VI are equal, the light

and dark velocities will be equal. Putting equation (4) equal to (13) and solving, gives

$$KI/2 = x^n/(a - x)^m \tag{14}$$

which, except for the number 2, is identical with the stationary state equation (5). This is the basis for Talbot's law (Talbot, 1834; Hecht and Wolf, 1932; Arnold and Winsor, 1934). The number 2 in the denominator depends on the equality of light and dark periods; the number to be used for any other ratio of periods is always the reciprocal of the fraction which the light duration is of the total light and dark cycle.

The effect of light on the photosensitive material is carried forward by the secondary dark reaction which the photoproducts undergo. According to equation (14) the concentration of these photo-products is x; therefore the rate of the secondary dark reaction is

$$(\Delta x/\Delta t)_{\text{secondary}} = k_3 x \tag{15}$$

where k_3 is a velocity constant. For the critical disappearance of flicker suppose that $\Delta x = c'$; that is, that the change Δx in concentration in the dark time Δt is just too small to cause the physiological change corresponding to a change in sensation of brightness. As in intensity discrimination this may mean a change in discharge frequency of the cell (*cf.* Adrian and Matthews, 1927; Hartline, 1934). The critical fusion frequency is f cycles of light and dark flashes per second; thus $f = 1/2\Delta t$. Substituting these values of Δx and of Δt in equation (15) we get

$$f = cx \tag{16}$$

where $c = k_3/2c'$. In other words, the critical fusion frequency is directly proportional to the concentration of photoproducts in the stationary state as given by equation (14).

The simplest four varieties of equation (14) are shown in figure 29. It is apparent that the value of n determines the slope of the steep limb of the curves, whereas that of m controls the curvature of the bend joining the steep limb with the horizontal one.

Examination of the data in figure 21 and in figure 23 shows that the rod curve always has twice the slope of the cone curve. This determines the value of n in the two cases; $n = 1$ for the rods, and $n = 2$ for the cones. The best curve to fit the cone data always has $m = 2$, and $n = 2$, as we just found also for intensity discrimination and for visual acuity. The rods, however, are somewhat variable with regard to the

value of m. This is illustrated by figure 30 which contains the single measurements of two individuals (Hecht and Smith, 1936) with a 19° field and with white light. Besides showing the adequacy and reproducibility of the data, especially in relation to the theoretical curves, figure 30 indicates this systematic variability of the rod measurements. Of the six runs, the rod data of four are described adequately by equation (14) only when $m = 2$, while the two others are better fitted by $m = 1$. The rod measurements of Hecht and Verrijp (1933b) are also better fitted by far when $m = 1$. Figure 29 shows that when $m = 2$, $n = 1$, the curvature is more gradual; whereas when $m = 1$, $n = 1$,

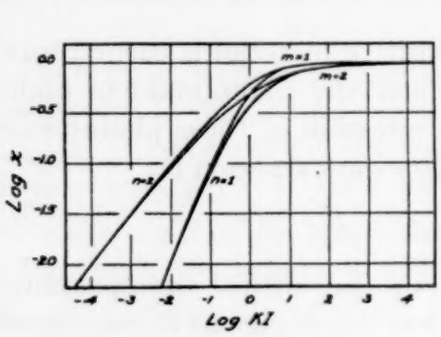

Fig. 29

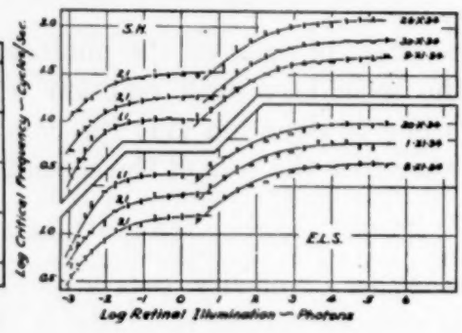

Fig. 30

Fig. 29. The steady state equation (14) plotted when m and n are each 1 and 2. Because of the log plot the shape of the curves remains constant regardless of the values of K and a.

Fig. 30. The single, individual measurements of flicker as taken in the course of a run (Hecht and Smith, 1936). For convenience the separate runs (dated to the right) have been spaced 0.2 log unit apart on the vertical axis; the values on the ordinate scale refer only to the topmost run for each investigator. The numbers attached to the rod curves indicate the values of m and n in equation (14) used in drawing them. The curve through the cone portions of the data is always from $m = n = 2$.

the transition between the rising limb and the plateau is sharper. Also, the plateau itself continues to rise gently in the 2,1 curve, whereas it flattens off quite rapidly in the 1,1 curve.

Whether these differences really represent daily variations in the state of the rod photoreceptor system, it is hard to say. The consistency with which either one or the other type of curve appears is, however, impressive for us who have watched them for many months.

e. *Other functions.* It would be surprising if all the basic data of vision and photoreception could be described quantitatively in terms of so simple a system of ideas and equations as has been used for the three functions discussed in this section. It is true that the data of several

other properties have also been fitted by this mechanism (Hecht, 1934a). But most likely it will not be possible to fit all the data into such a simple system, because the reactions are certainly more involved than I have supposed.

The primary light reaction is probably simple enough, as many primary photochemical reactions are; but even with visual purple recent careful work shows complications, such as a dependence on pH (Chase, 1936), and a dark after-bleaching (Hosoya, 1933). The primary dark reaction probably also involves more than the direct reconversion of photoproducts into sensitive material, since Wald (1935b) has confirmed Kuehne's idea that visual purple may be formed from its products in two ways,—directly, and by the addition of new material,—and has shown (Wald and Clark, 1936) that dark adaptation brings these into evidence under certain conditions.

The important thing about the stationary state equation (5) is that, in spite of the simplicity of its derivation, it describes the relevant data with excellent precision, showing that fundamentally its simplicity is sound and that in the main its ideas are basic in the photoreceptor process. Naturally, complications are present. We hope soon to publish new measurements of dark adaptation (Hecht and Haig, 1936) which can be fitted theoretically only by a slightly more elaborate treatment. However, no matter what the complications are, the equations which describe them must reduce to the stationary state equation (5) since this equation describes the straightforward data of intensity discrimination, visual acuity, and flicker. Whatever else has to be added only serves to define more explicitly the nature of the photoreceptor process.

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FORMATION AND DESTRUCTION OF RED BLOOD CELLS

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The present article supplements the reviews of the work on red blood cell formation and destruction summarized in Physiological Reviews in 1923 by Rous (1), in 1922 and 1928 by Sabin (2–3), in 1929 by Robscheit-Robbins (4), and in 1936 by Ponder (5). Present data substantiate some of the points which the earlier literature suggested, throw new light on others, but leave a number of the problems still unsolved.

The most recent advances in our knowledge of red blood cell formation have developed from the work on pernicious anemia. The evolution of the present attitude will be best understood if the articles are reviewed in the order in which they influenced contemporary views.

The increased study of the bone marrow by biopsy and at autopsy has emphasized the fact that the tissue is very labile in that the numbers and percentages of cells at different stages in development vary widely in health as well as in disease (6–14). It has thrown the emphasis from the blood stream to the marrow itself, and has served to clarify many clinical conditions. Briefly the stages in the development of the erythrocyte are *a*, a primitive blast (called by various names); *b*, a megaloblast (not recognized as a stage in normal development by all authors); *c*, a large or macronormoblast or basophilic normoblast; *d*, a small or eosinophilic normoblast; *e*, reticulocyte (young, with much reticulin substance; old, with little reticulin substance); *f*, granule red blood cell; *g*, adult erythrocyte (young, relatively resistant to heat and hemolysis; older form, less resistant). The cells mature to the reticulocyte stage in the bone marrow and then enter the blood stream. About 1 to 3 per cent of the cells in the normal peripheral circulation are in the reticulocyte stage. It is not known whether these represent the normal increment of young red blood cells at any given time in the blood or whether their appearance is fortuitous. There is a difference of opinion among authors as to whether immature cells, entering the blood stream can continue with their maturation process in the new environment (15–16). It is not difficult to imagine a reticulocyte

losing its acid substance in the relatively alkaline and well buffered plasma, but this very fact makes it seem unlikely that an immature nucleated cell would continue its normal metabolism under the same circumstances.

Development of the concept of specific stimuli. Several observations have added considerably to our knowledge of blood formation and have served as a new stimulus to research in hematology. The first were the experiments of Whipple, Hooper, Robscheit-Robbins and their collaborators (17–20) in demonstrating that hemoglobin formation was definitely influenced by specific food factors. Whipple and Robscheit-Robbins worked primarily with dogs whose hemoglobin stores were depleted by chronic hemorrhage. The second important contribution was that of Minot and Murphy (21), who demonstrated that liver contained a substance which acted favorably in pernicious anemia. Minot (22–24) recognized the characteristic increase in the number of reticulocytes after adequate therapy (the "reticulocyte response") and this initiated the study of the concept of a specific stage in the development of red blood cells (the megaloblast) being sensitive to a specific substance (liver extract). This was given additional foundation when Peabody (25), corroborating earlier work (26) in studying the bone marrow in pernicious anemia, demonstrated an accumulation of cells in the megaloblast stage during relapse, with a decrease in number, associated with a reticulocyte increase, during a remission induced with liver therapy. Other work has served to give more specific data. Thus while normal sternal bone marrow contains from 25,000 to 35,000 megaloblasts per cu. mm., in pernicious anemia the number may vary from 60,000 to 112,000 per cu. mm. (27). It seems evident that the red blood cells develop from a primitive form into a megaloblast stage, a normoblast stage (large or macro-normoblast and small or eosinophilic normoblast), a reticulocyte stage, a granule red blood cell stage and finally the mature erythrocyte. At this stage the work of Castle (28–31) demonstrated that the origin of the megaloblast-maturing substance was associated with gastric digestion. He showed by a clear cut series of experiments that an "intrinsic factor" in the gastric secretion (presumably an enzyme, from the nature of its properties) interacts with an "extrinsic factor" (something in the food, probably protein, possibly vitamin B) (32). The material which is produced is not identical with liver extract, but is apparently an intermediate stage in its production (33–35). It was then demonstrated that the material existed in the gastric cells (36–41) predominantly in the pyloric region (42).

The stages in the development of the red blood cell. The series of observations cited above made it evident that megaloblasts mature into normoblasts and eventually into reticulocytes and adult red blood cells, but left unanswered the problem as to whether megaloblasts in pernicious anemia were atypical cells, similar to specific embryonic erythrogenic cells, but not found in the normal adult individual (43), or whether they were normal stages in the development of red blood cells (44). Certainly the blood of the fetus does have many of the features of that of pernicious anemia, namely, macrocytosis, nucleated forms, and low red blood cell count (45). Of interest in this connection is the fact that in some patients with cirrhosis of the liver and with sprue, the red blood cells are larger than normal (46–49). Bone marrow studies (49, 50) showed here, as in pernicious anemia, that there was a relative inhibition at the megaloblast stage. This is presumably due to failure of the liver to store, elaborate or otherwise metabolize the hematopoietic substance (51) and consequently it was not available for adequate use in the bone marrow. Therapy with liver extract restores the blood and bone marrow to normal (50). The picture can be produced experimentally in rats by treatment with carbon tetrachloride (52). From these experiments it is evident that a "megaloblastic" bone marrow can develop in a non-pernicious anemia individual, and second, delay at the megaloblast stage results in an increase in the size of the red blood cells which eventually mature from these cells. Presumably the stroma is built up in the megaloblast stage, while most of the hemoglobin is manufactured in the subsequent stages.

Further confirmation of the relationship between the megaloblast and reticulocyte stages is shown in the remarkable mathematical relationship between the degree of anemia, in pernicious anemia, and the number of reticulocytes produced after liver or stomach therapy. The greater the inhibition of cells in their development at the megaloblast stage, the greater the anemia and consequently the greater the number of reticulocytes produced when the "stored" megaloblasts are simultaneously supplied with the substance which stimulates them to mature. When but few cells are inhibited, there is but little anemia, and coincidentally but few reticulocytes are produced at any one time. This may be expressed mathematically by the equation $R = \frac{0.73 - 0.2\ Eo}{0.73 + 0.8\ Eo}$ when liver extract is taken by mouth; $R = \frac{91.6 - 25.9\ Eo}{1 + 0.57\ Eo}$ for desiccated stomach therapy and $R = \frac{93.7 - 24\ Eo}{1 + 0.47\ Eo}$

for intravenous liver extract. (Eo = initial red blood cell count in millions per cu. mm.; R = maximum reticulocyte percentage) (16, 22, 23, 52).

Expressed in another way, the total reticulocyte production under these circumstances is inversely proportional to the initial peripheral red blood cell level. While this cannot be measured directly, an indirect measure is the area of the space surrounded by the daily reticulocyte curve (based on the absolute number of cells) and its base line when plotted under standard conditions on graph paper. This area is inversely proportional to the initial peripheral red blood cell count according to the formula, $Area = \frac{67.37}{Eo^{.385}}$ sq. cm. for liver extract by mouth and $Area = \frac{86.93}{Eo^{.385}}$ for desiccated stomach. (Eo = initial red blood cell count in millions per cubic millimeter at the beginning of the treatment) (54).

Release of cells into circulation. The mechanism of the release of the red blood cells into circulation is still a matter of study. The view that postulates temporarily closed, intersinusoidal capillaries with red blood cell formation within their lumen, pictures the mature erythrocytes entering the circulation by the reopening of the capillaries as part of the general circulation. This view would require the capillaries to open and release their mature cells, and then close for further cell formation (55). Others (56–60) consider that the red blood cells are formed in the parenchyma of the bone marrow, and are released by diapedesis or by temporary dissolution of the capillary walls. Under the latter condition immature cells are described as being surrounded by a thick intercellular jelly-like substance, which liquefies as the cells reach maturity. Cells then pass from liquid to liquid, through the capillary walls (57). Both concepts consider the bone marrow circulation as being of the "closed" type, as opposed to the idea of capillaries opening up into "tissue spaces" (55–60).

The primitive erythroblast. The stages of growth before the megaloblast have led to various theories. The cells are round or oval and have nuclei measuring on the average, 7.5 x 10 microns in stained films. The cytoplasm is very basophilic, and is not distinctly organized, except in the later stages, the earliest phase being that of a syncytium. The cells look something like lymphocytes on superficial examination, but differ from them grossly on detailed study. Their chromatin is finely divided; nucleoli are prominent; their nuclei are much larger than

those of small lymphocytes. They have been classified as lymphocytes by some (61), lymphoid cells by others, a third group consider them really primitive forms of myeloblasts (62), while a fourth group consider them as specific red blood cell blasts.

Stimulus to erythrogenesis. The stimulus to red blood cell formation is not clearly defined. In aplastic anemia, the cells are blocked or inhibited at the primitive blast stage; in pernicious anemia, at the megaloblast stage; in other anemias at the normoblast stage. It is quite definite that when missing materials are supplied in adequate amounts, as liver extract in macrocytic anemias, or iron, in iron deficiency anemias, the stimulus to proper growth is evident (63). So far, no specific stimulus is known which will make megaloblasts reproduce more rapidly in aplastic anemia. In experiments with pigeons, leucine and lysine caused a "spread" of the bone marrow, presumably causing more rapid cell growth (64). Elevation in the temperature of the bone marrow appears to have a similar effect (65). Certain substances cause an increase in the number of reticulocytes in the blood stream, either by suddenly increasing the rate of maturation of normoblasts, or possibly by causing a decrease in mature cells with compensatory reticulocytosis. To this group belong iron (63–66), vitamin C (67), arsenic, and possibly certain amino acids or other organic agents. These are classified as "non-specific reticulocytoses" as opposed to the type produced by liver extract or desiccated stomach.

After hemorrhage, acute or chronic hemolytic phenomena, or exposure to low oxygen tensions (68–70), there appears to be some type of stimulus which causes red blood cells to be produced at a more rapid rate than normal. Two factors are evident: first, a more rapid maturation of red blood cells so that, having hurried through the megaloblast stage, they are smaller than normal, and having passed through the normoblastic stages rapidly, they are poor in hemoglobin. Second, there must be more rapid proliferation of the blast cells, as the marrow tends to spread in the long bones and displaces the fat. As to the nature of the stimulus, whether it is the result of low oxygen tension in the tissues, high carbon dioxide content, or a vasomotor phenomenon, there are no consistent data. In pneumonia there may be low oxygen and high carbon dioxide content of the blood without reticulocytosis (71, 72). Polycythemia may be associated with defective pulmonary ventilation (73, 74) or thickened bone marrow blood vessels (i.e., defective oxygen interchange) (75). Yet in generalized arteriosclerosis, in hypertension and hypotension, there is no characteristic evidence of

abnormal stimulation to hematopoiesis. Another feature which must be considered is that in pathological conditions the predominant stage of development of red blood cells may differ in different bones in the same patient, suggesting a local, rather than general effect of the stimulus. In aplastic and hypoplastic anemias, the rate of red blood cell formation is extremely slow, regardless of any stimulus which may arise from the need of new cells, and the few cells which do reach the peripheral circulation are normal in size and shape.

Shape. There are very little data which would help one to understand how the red blood cells develop or maintain their shape. While under normal circumstances, in man, they are round or almost round (76) biconcave discs, perfectly healthy individuals appear with oval red blood cells (77–80) or other curious forms (sicklemia) (81, 82). The macrocytes in pernicious anemia are predominantly oval, while those in sprue, cirrhosis of the liver and other conditions are round. This may be evidence that the megaloblasts in pernicious anemia are really pathological cells, and differ from the precursors of normal red blood cells. In the anemia of chronic hemorrhage "pencil" shaped red blood cells may appear (long axis 3 to 5 times the width). Poikilocytosis has been considered as an evidence or stage of the destruction process, without, however, adequate proof. The extremely small type of microcytes may be fragmentation products (83). From reported data the type of fluid in which the corpuscles may be suspended experimentally (84), the acid and base content, the pH of the medium, the osmotic pressure, as well as specific ions, determine some of the features of the shape, size and properties of the corpuscles. In "hypotonic" solutions, human erythrocytes tend to become spherical (85). Distortion of the cells follows the addition of lecithin, cholesterin (83) or alanine (86) to the blood.

Red blood cell destruction. In the present state of our knowledge we still are unable to say how aged red blood cells are normally disposed of, or even the exact place. Phagocytosis, fragmentation and hemolysis occupy the same relative importance today that they did when Rous compiled his review in 1923. Possibly more emphasis is being placed by some authors on the so-called "reticulo-endothelial system"—a vague and miscellaneous group of cells, as a source of phagocytosis and bilirubin production. Unquestionably phagocytosis of red blood cells does take place in some cells. The ingested corpuscles become smaller and paler in the cytoplasm of the engulfing cell until finally a small amount of granular debris remains. In some cells pigment

deposits accumulate (hemosiderin), especially after the intensive blood destruction following transfusion. Occasionally phagocytic cells enter the peripheral circulation (subacute bacterial endocarditis or after blood transfusion). These cells have a superficial resemblance to the monocytes of the blood stream, but in reality are cytologically entirely different (shape and structure of the nucleus; architecture of the cytoplasm (87)).

The function of the mechanism of destroying red blood cells within the cytoplasm of phagocytes appears to be the conservation of the products of hemoglobin decomposition, and the conversion of the pigment into hemosiderin (88, 89), bilirubin or similar substances. The process has been studied in tissue cultures (90). Hemolysis within the blood steam is followed by excretion of hemoglobin by the kidneys as is noted in paroxysmal hemoglobinuria and after certain blood transfusions in which there is a strong hemolytic element or when hemoglobin is injected intravenously (91). It is problematical as to what test tube hemolytic phenomena are operative under the conditions of phagocytosis.

The ordinary "fragility test" for resistance to hypotonic sodium chloride solutions suggests that in ordinary blood some cells hemolyse more easily than others. According to some evidence the most resistant cells are the young ones (92–96), but all authors do not agree to this idea (97–102). This suggests, however, that certain cells have undergone changes which make them hemolyze more easily than other ones when exposed to hypotonic salt solutions in a test tube. If these cells are the first to be destroyed in the body, presumably this change is associated with some property of the cell which causes it to be phagocytized easily, as there is no reason to believe that it will meet with a hypotonic sodium chloride solution in the body.

It seems logical to assume that hemolysis plays a part in normal red blood cell destruction, yet there are no direct data demonstrating this. Under abnormal conditions, the globular red blood cells in congenital hemolytic jaundice may be hemolyzed more readily than the biconcave discs of the normal individual, the globular form being one step nearer hemolysis than the flattened disc (103). Yet after splenectomy, evidence of abnormal hemolysis disappears, although the congenital shape of the red blood cells appears unaltered (104, 105). The spleen must play a part, yet after splenectomy, polycythemia from accumulation of cells does not develop.

The nature of the hemolytic process is not definitely known. Ex-

tensive studies of the physical and chemical laws of hemolysis *in vitro* have been reported (5, 106, 107), but little is known concerning the process in the body. A system based on osmosis, but modified to explain the behavior of the red blood cells to physical and chemical agents is favored by some (106). The colloid chemical aspects of hemolysis are advocated by others to explain hemoglobin adsorption, release, swelling of the corpuscles and readsorption (108, 109, 110), without assuming an osmotic system on a "sliding scale" to explain varying behavior toward different substances.

Certain of the pathologic forms of blood destruction may give a clue for further work on the physiologic processes. In lead poisoning there is some evidence that the surface of "leaded" red blood cells is different from normal, and that these cells change from "an elastic distensible sac, to one which is contracted, relatively inelastic and brittle," with relatively poor ability to withstand trauma (111). In malaria there is presumably a disruption of the red blood cell, with release of hemoglobin into the blood plasma. The mechanism of phenylhydrazine blood destruction is not clear, as hemolysis does not take place *in vitro*, when blood is mixed directly with this reagent. Similar effects are produced by many chemical agents. In paroxysmal hemoglobinuria, hemolysis follows exposure to cold under conditions which suggests that a hemolytic substance is liberated into the blood plasma. The spherocytosis of congenital hemolytic jaundice, with a hyperactive spleen has been mentioned. Fragmentation of the red blood cells has been observed in the peripheral blood in erythroblastic anemia (112) and other conditions (113).

It is possible to generate serums which hemolyze red blood cells, and this mechanism may account for the hemolytic phenomena after certain blood transfusions, especially after the blood of one donor has been used repeatedly in the same patient. Under these conditions hemoglobin appears in the blood plasma and much of it is excreted.

These types of hemolysis, under pathological conditions may however involve normal processes and therefore serve as means of studying physiological red blood cell destruction.

Length of life of red blood corpuscles. The length of life of the red blood cell is not known. Observations on the use of liver extract in pernicious anemia suggest that it takes about 7 days (4–11 days) for the megaloblast to mature to the reticulocyte stage (66, 114, 115). It probably takes about two days for the youngest reticulocyte to mature into an adult red blood cell. It has been estimated that reticulo-

cytes with moderate to heavy reticulation, in the peripheral blood in pernicious anemia after liver extract therapy, have been liberated into the blood during the preceding 24 hours, while those with faint reticulation were present in the blood for 24 to 48 hours (16). This assumes that reticulocytes undergo progressive maturation in the blood stream. Others place the duration from 1 (72) to 4 days (116).

TABLE 1

AUTHOR	METHOD	DURATION	ANIMAL
Addis (117)	Bile pigment production	2 weeks	Man
Todd and White (118)	Specific exhausted isohemolytic serum	4–7 days	Bulls
Schulten (119)	Regeneration after severe anemia	3 weeks	Rabbits White mice White rats Man
Ashby (120)	Selective agglutination	3 months	Man
Wearn, Warren and Ames (121)	Selective agglutination	59–113 days	Man
Jervell (122)	Selective agglutination	12 hours to several weeks	Man
Eaton and Damren (123)	Periods of regeneration	8–8.5 days 16 days	Rabbit Dog
Isaacs (124)	Periods of regeneration	6–8 days	Man
Isaacs (124)	Transfusion in aplastic anemia	5–27 days Aver. 14 days	Man
Kindred and Corey (125)	Persistence of small cells	4–5 days	Rat fetus
Escobar and Baldwin (126)	Response to change in oxygen pressure	12–18 days 16–23 days 18–30 days	Rat Dog Man
Wiener (127)	Selective agglutination	80–120 days	Man

For the adult erythrocytes, estimates of life duration depend on the method used. Some of the observations are summarized in table 1.

In the data in table 1, many factors enter to qualify or modify the conclusions. In the bile pigment formation method, other sources of bile pigments must be taken into account. In pernicious anemia it is possible that some of the bile pigment in the blood stream may be the result of "unused" pigment precursors, the result of the failure of the cells to mature (128). In the experiment with anemic animals or men, the problem of the identity of conditions with those in health

must always be considered. In the work on agglutination of transfused cells, the fact that immature cells may simulate the transfused cells in their non-agglutination properties is often overlooked (129). This method is not reliable after 2 to 4 days. In all the reported data the estimates of the life of adult erythrocytes vary from 4 to 120 days in various animals and man.

A source of endogenous uric acid in the body may be the discarded or destroyed nuclei of the growing red blood cells (130, 131, 132). The amount of uric acid excreted during the "reticulocyte response" period in induced remissions in pernicious anemia, in excess of the normal excretion, is proportional to the increase in the number of red blood cells. Approximately 10 grams of uric acid are excreted in the urine with the increase of a million red blood cells per cu. mm. in the blood (16). The increase in uric acid in the blood starts within 24 hours after the onset of treatment and the "peak" production precedes the "peak" in the reticulocyte production by about 24 hours (132). These data offer material for speculation as to the normal rate of destruction of red blood cell nuclei, and consequently the rate of formation of red blood cells, as well as the length of time that it takes for megaloblasts to develop into normoblasts and to discard and decompose their nuclei and mature to reticulocytes.

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THE NATURE OF UTERINE CONTRACTILITY

A Survey of Recent Trends

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PERIODIC RHYTHMIC MOTILITY AND THE OESTROUS CYCLE. *Description of movements.* One of the first accounts of the cyclic variations in uterine and tubal movements contains the statement which best summarizes the current views regarding them (39). There, Corner states that when a segment of uterus was suspended in physiological salt solution, it was found "that when the uterine tissue was taken from a sow killed during oestrus, the contractions were large and slow (1.5–2.5 min. apart), but during the first few days following ovulation the contractions became more rapid and less ample until, at the tenth day, they were occurring at the rate of 8 per minute, but excursions of the writing point were not more than one-fifth or even one-tenth of the previous magnitude." This view is accepted today as being generally applicable to most forms so far studied, but it may be subject to modification, as Corner points out (39), when the different types of oestrous activity are considered.

These cyclic changes were first established in the rat (21, 22) and sow (102) and have been amply confirmed (206, 38, 68, 49, 116, 80, 184, 32, 75) and extended to include a variety of other species which exhibit cyclic ovarian activity and which ovulate spontaneously (mouse, 99, 188; guinea pig, 188, 32, 86; opossum, 83; cow, 192, 54; dog, 14; human, 110, 182, 204, 89; monkey, 201, 202; reviews, 90, 2, 141, 172). In addition, major rhythmic contractions are found during the persistent oestrus in the three species having this type of ovarian activity which have been studied thus far (rabbit, 161, 200, 148, 145, 205, 133, 23; cat, 64; squirrel, 63), whereas much less motility is associated with the early phases of pregnancy or pseudopregnancy which these forms undergo when ovulation takes place after the normally adequate stimulus of coitus. Broadly speaking, therefore, in all the species thus far studied, typical uterine motility of the kind described at the outset is found coincident with the growth and influence of Graafian follicles (i.e., during pro-oestrus and oestrus).

While it is generally agreed that there is a myometrial motility cycle, there is, however, a lack of agreement as to all of the characteristics of this motility cycle. To be sure, general unanimity of opinion exists concerning the type of motility found at oestrus (major rhythmic contractions), but there is less agreement about the motility which is associated with phases of the sex cycle other than at oestrus. Four investigators report (49, 184, 14, 134) a mere increase in the frequency of the contraction rhythm during dioestrus, with little change in the amplitude of the recorded contractions. Clark, Knaus and Parkes, however, find no certain relationship between the frequency of uterine contractions and the oestrous cycle (38), while other investigators stress an acceleration in the frequency of contractions associated with a marked diminution in amplitude. As a result, motility of an undulatory character is found (39, 102, 22, 206, 83, 68, 82, 75). Still other observers stress the virtual quiescence of the uterus in the rabbit, cat, squirrel and monkey during anoestrus or pregnancy (107, 200, 202, 63, 64, 161, 162, 148, 146, 145, 23 and others) in contrast to the mere *change* in contraction frequency found in other species. These apparent discrepancies must not be dismissed and ought to be explained. Recently, Pompen (145) has reported observations which may explain in some measure the present conflicting data. This work emphasizes, as the special feature of anoestrous motility in the rabbit, a lack of coördination in the various contracting portions of the uterus as contrasted with the high degree of coördinated motility of the uterus as a whole during oestrus. Similar observations have been made by Westman (202, 200). These findings are descriptions of activity of the whole uterus *in situ*, and as such, they are observations with which the results of all graphic methods of study must be harmonized eventually; contrary findings indicate the presence of artifacts or inadequacies of technic.

Perhaps some of the differing viewpoints are capable of explanation on the basis of variations which the several technics impart to the records which are obtained. Nevertheless, Knaus (108) concluded that data obtained by him by means of *in vitro* and *in vivo* technics are for the most part comparable. In a more recent investigation of this subject by Robson (176), it is found that there is some agreement between the data of *in vitro* and *in vivo* technics. Robson concludes that the findings of the two methods are comparable, but he also says: "With regard to the movements of the circular muscle *in vitro* as compared with those *in vivo*, such marked variations occurred that the significance

of the data is difficult to interpret, and it seems possible that variations due to internal pressure have not been sufficiently investigated in the excised preparation." Other investigators have also commented upon the differences between the various results from *in vitro* and *in vivo* experiments (195, 23, 199, 180). Inasmuch as some methods record changes in length of long strips of longitudinal muscle, others record from short lengths, and still other methods record combined effects of longitudinal and circular muscle (as in balloon recording), it is apparent that generalizations must be drawn cautiously when reliance is made upon data from any one type of experimental procedure. Further consideration ought to be given, in the future, to the characteristics which various procedures impart to the kymographic records of myometrial activity. Painter and Harne have made a notable beginning in this respect, by recording simultaneously the changes in longitudinal and circular muscle, along with intra-uterine pressure changes (139). Gruber has likewise studied the technical difficulties involved in the balloon method of recording (77; see also 133, 146).

The results from such studies may not in the end alter the views which are now held largely by inspection alone, but they will serve to unify a body of data which are at present conflicting into a harmonious whole that will have an etablished basis of fact. This, after all, is the desideratum of scientific experimentation. It is to be hoped that quantitative methods of studying uterine contraction and relaxation will be developed so that one may appreciate the significance of all the various contraction forms which have been observed. Without precise methods for studying heart action the cardiologist would be at a loss in his study of the dynamic events of the cardiac cycle and, likewise, the student of the physiology of reproduction will be compelled some day to regard uterine motility in a similar quantitative manner in order to appreciate fully the dynamic aspect of uterine contractility.

Summary. The chief established facts concerning periodic uterine motility are as follows: at pro-oestrus and early oestrus, leisurely rhythmic contractions of considerable force and amplitude occur, as may be seen in the intact, unoperated uterus as well as with certain other modes of studying uterine contractility. The physical characteristics of this rhythmic motility are imparted by a coördination of otherwise uncoördinated movements in the various portions of the uterus. As a consequence, this coördination gives timed unity of action to the different portions of the uterus, so that these contractions become, on well-known physiological grounds, more effective and force-

ful for the functions they subserve. The implications of this fact will be discussed below. During anoestrus and dioestrus, coördinated contractility of the whole uterus is no longer found, since the oestrous type of motility becomes less distinct owing to the independent activity of various parts of the uterus. More frequently occurring and less ample contractions are usually observed, with the result that they are less forceful and effective than are those occurring at oestrus.

Correlation of uterine activity with ovarian activity. Associated with increasing follicular activity, one finds the oestrous type of motility described above while in the dioestrous phase of the cycle undulatory uterine and tubal movements have been related to the sojourn of the ova in this portion of the genital tract (39, 102, 206, 161, 200, 116, 203, 75, 54; reviews, 39, 84, 140). Thus, the latter type of activity has been associated with waning of follicular activity, the beginning of corpus luteum function, and the migration of ova (cf. 39). A number of investigators, however, have not attempted to make so close a correlation of the various types of motility with ovulation and the migration of the ova through the tubes and into the uterus; rather, they have noted only the major rhythmic contractions of oestrus and smaller, more frequent ones in dioestrus and pregnancy (83, 68, 38, 32, 49, 86, 63, 64, 14).

While in the main confirming the above findings obtained principally with the uterus of the mouse, rat, sow, opossum, guinea pig, cat, dog, and squirrel, several investigators have made more precise chronological records of the changes which occur in the uterus of the rabbit as certain specific and well-known events take place in the ovary. The findings are interesting in view of the light they throw on the importance of motility records made with one and the same uterus over extended periods of time (200, 161, 145, 133, 23, 201, 146). Such observations made both with the abdominal window and with the uterine fistula in unanesthetized rabbits show that the post-coital change from the rhythmically contracting organ to the virtually quiescent one is relatively abrupt. Thus in the rabbit, which ovulates normally about ten hours following coitus or other suitable stimulus, the contractions nearly disappear some five hours or more *prior* to ovulation. This state of uterine quiescence remains unchanged for nearly the duration of a period of pseudopregnancy or pregnancy, at the end of which rhythmic, major contractions gradually reappear (161, 133, 145, 23, 200). Accordingly, in the rabbit at least, it is not sufficient to say that motility of the oestrous type is lacking during pseudopregnancy or pregnancy,

inasmuch as the change comes even before the ovum is discharged from the ovary, and so at a time when pregnancy may hardly be said to have begun. It is clear, therefore, that some preovulatory influence brings about an inhibition of coördinated uterine contractions in the rabbit.

Is this influence ovarian or extra-ovarian in origin? Does it occur in other species or only in those which ovulate in response to the stimulus of coitus? And is it possible, as Fellner suggests (58), that the difference in endocrine control of the two types of reproductive cycle (e.g., cyclic, as in the rat, and acyclic as in the rabbit),—is reflected in the motility characteristics found in the two species? It may be significant in this respect that Evans and Miller, recording in unanesthetized cows, find no pre-ovulatory cessation of motility (54), so confirming the indirect data obtained on excised sows' uteri (39). And although, as Hartman points out (85), the endocrine regulation of bodily activities is in a functional sense as constant a characteristic of vertebrates as is the notochord in a developmental sense, nevertheless well-known species variations in reproductive activity are so prominent that Fellner's suggestion may possibly be regarded as a working hypothesis for experiments designed to reveal the nature of the underlying mechanisms involved in the two types of sex cycle, viz., cyclic and acyclic.

Correlation of uterine cyclic activity with uterine functions and with sex behavior. The principal aspect of tubal and myometrial activity which has so far occupied the attention of investigators has been the relationship of diminishing pre- or post-ovulatory uterine activity, as described above, to the time required for passage of the ova down the tubes and spacing them in the uterus. Little attention has been paid to the functions that are served by the most prominent feature of cyclic uterine motility, namely, the major contractions that are found at oestrus.

These contractions are not necessary for libido in the infra-human animals since this is unaffected by total hysterectomy (48, 44) and since rabbits will receive the male during pregnancy, at which time there are no major contractions (161, 145, 133, 107, 157, and others). Moreover, hysterectomy does not affect the well-known periodic increase of bodily activity which is associated with the oestrous cycle (48) and is attributable indirectly to the action of oestrin (35). Loeb first observed that hysterectomy in the guinea pig modifies the function of the ovary, but there is no evidence to show that a loss of uterine motility is in any way concerned in this effect. He suggests that a hormonic factor is involved (123). Moreover, the best of current opinion indi-

cates that major uterine contractions are not essential for migration of spermatozoa in their ascent to the uterine tubes (84, 140): direct evidence by Parker is against this view and indirect evidence by Hammond shows that sperm can ascend the uterus and tubes at a time when these structures are quiescent. Thus Hammond observed that a rabbit may become pregnant when it is inseminated at the time of experimentally-induced ovulation (79), at which time, as mentioned above, the uterus is quiescent. Major uterine contractions are not, therefore, an essential part in the mechanism of the migration of spermatozoa in the uterus. Parker maintains, on the other hand, that uterine contractions are chiefly responsible for entry of spermatozoa into the uterus through the cervices (140), although he adduces no direct evidence in support of this contention, and attempts by Walton to obtain data on this point by direct experimentation have yielded negative results (198).

As these more or less apparent functions are seen to be served without the aid of marked uterine contractility, the question arises: What real function or functions may be served by rhythmic, major contractions? Or are they merely a functional embellishment of every oestrous cycle? Evidence bearing upon the answers to such questions will form the last principal part of this review. For the present, attention will be directed to a brief resumé of the available facts concerning the hormonic control of cyclic uterine activity.

Hormonic regulation of periodic uterine motility. In view of what has been said above it is hardly necessary to mention that the myometrial cycle, like the well-known structural cycle found in the endometrium, is under the control of the ovary. This fact, however, has not been long established.

The first conclusive evidence indicating the dominant influence of the ovary on uterine motility is found in the castration-transplantation experiments of Athias in 1919 (12). Athias confirmed earlier observations (cf. reviews, 124, 130) on the effect of ovariectomy when he found that following this operation rhythmic spontaneous motility of the uterus is lost. He then extended these observations by showing that motility may be restored to the uterus when ovarian grafts are made in the ovariectomized guinea pig even though the animal may have been deprived of its ovaries for more than a year. Thus the replacement effect of the ovary was found to be complete and at the same time a significant relationship between ovarian function and uterine activity was established. This finding is, of course, conso-

nant with the well-known structural involution which takes place in the uterus as a result of ovariectomy, as shown many years before (cf. 130) and amply confirmed on numerous recent occasions. It should be noted, however, that there is no intimation in the work of Athias of the periodic nature of uterine motility in the guinea pig although the cyclic vaginal changes in this animal were described two years earlier by Stockard and Papanicalaou.

In contrast to this and earlier work showing the occurrence of uterine quiescence following ovariectomy, one finds recent reports which indicate that uterine muscle has an inherent rhythm of contractility which is merely *modified* by hormonic influences (82, 137, 193, 134, 133, 14; cf. 172), but such reports are at variance with other experiments which show that in the absence of the hormonic action of oestrin, the uterus becomes non-motile (148, 23, 145, 170; cf. 124).

The reason for the opposing views is not clear. It is possible that some basis for the divergent conclusions is attributable to variations in the use of technics which are described as similar. An example of this is found in the observed effect of ovariectomy in unanesthetized rabbits and dogs as reported by three investigators (148, 133, 15). In one case it was found that quiescence of the uterus inevitably follows complete castration, even more rapidly than uterine involution (148; see also 75) whereas in the hands of the other investigators, Morgan and Azuma, the uterus was found to remain active, but the contractions occurred with a frequency and form (as observed in the published records) which differed from that found at oestrus. Morgan gives no information concerning the length of time such motility will continue in the ovariectomized animal, nor does he mention the balloon size or the tension under which it is usually distended in his experiments. Azuma employs a balloon of large size (10 ccm.) in relation to the size of the uterus, and a distending tension of 100 cm. of water. Data concerning these conditions are important in view of an observation which the writer, in collaboration with W. M. Allen, made as the result of an accident in a recent series of experiments on the motility-inhibiting property of the corpus luteum hormone, progesterone (7). In the course of an experiment the intrauterine balloon (distending tension of 18 cm. of water) became weakened on one side, bulged, and increased in capacity to several times the original size of 1.5 cc. As a result of the altered condition the contractions became greater and greater,—a result which contrasted markedly with more than four dozen similar but successful experiments in which the uteri became quiescent. Clearly, physical

relationships involving the capacity of the balloon, distending tension, and length of muscle fibers, are factors of prime importance in determining the characteristics of the recorded motility (cf. 77). In this particular experiment, at least, the altered physical conditions completely annulled the predicted hormone effect, but this is what one would expect on simple physiological grounds when the length of the muscle is increased. Such an observation also serves to emphasize the fact that caution must be exercised in evaluating the significance of uterine records both when they are obtained *in vivo* in unanesthetized animals as well as *in vitro*, with muscle strips of varying length and thickness and with different lever loads.

Summary. Broadly speaking, one may say that after ovariectomy the uterus shows little if any spontaneous motility unless resort is made to appropriate replacement therapy as will shortly be described. Thus the dependence of uterine motility upon one phase (follicular) of ovarian function is established. We shall now consider in detail the essential characteristics of the replacement effects of oestrin on the one hand, and of progestin on the other.

INITIATION AND MAINTENANCE OF RHYTHMIC UTERINE MOTILITY. *Oestrin.* The first experiments with oestrogenic extracts which were successful in initiating uterine motility were performed in 1925 (68). This work by Frank and his collaborators showed that administration of suitable extracts of proven oestrin content induce rhythmic motility after twenty-four hours when the uterus is tested *in vitro*. This finding has been amply confirmed on a number of recent occasions (170, 134, 96, 28, 176, 23, 75).

With the establishment of this fact it was natural that attempts should be made to initiate rhythmic uterine contractions with uteri of suitable (e.g., ovariectomized) animals by the addition of oestrin to a perfusion bath containing the excised tissue. These attempts fall in one of two categories: 1, experiments with crude oestrin-containing extracts, and 2, experiments with one of the various forms of crystalline oestrin (oestrone; oestriol).

In several investigations in which crude extracts were used, the myometrium was stimulated to activity (57, 120, 29), but the majority of experiments in this group yielded negative results (27, 66, 23, 96, 167, 67, 92) or showed inhibition of such motility as existed (49, 92, 47, 183). Experiments with crystalline oestrin have given uniformly negative results (134, 82, 47, 96, 23, 129, 170, 92). The reason for the ineffectiveness of pure oestrin administered *in vitro* remains to be elu-

cidated. Whether the failure is attributable to the very low solubility of oestrin in water and its consequent unavailability to the tissue (cf. 92), or whether the failure is ascribable to the inability of the excised tissue to utilize the hormone when the uterus is outside the body has never been determined (92). It is probable that essential nutritive elements are not supplied in the perfusion fluid of these experiments.

Special mention should be made of the accounts which, contrary to the majority of this type, report responses of the uterus when the hormone is added to the bath containing the excised tissue. In some of these experiments it is found that oestrin effects a diminution of such spontaneous motility as the uterus may exhibit at the time it is excised (48, 49). The meaning of these results is not clear since the inference to be derived is that oestrin serves only to decrease the frequency of the uterine contractions at the time of oestrus. This conclusion drawn by Durrant and Rosenfeld is contrary to the preponderance of evidence from similar and other technics which shows that an increase in the amplitude and coördination of the contractions is generally found to be the most significant characteristic of cyclic uterine motility. The results of the experiments performed by Durrant and Rosenfeld are clear enough, but their meaning in terms of normal function is not.

A second group of experiments by Brouha and Simmonet (28, 29, 30, 31, 32, 33) deserves special comment since the observations of these investigators are widely quoted in reviews on the subject of oestrogenic hormones (2, 3, 90, 141) and since the data of these experiments are of a positive nature. In the first place the results of these investigators show that an agent essential for rhythmicity of activity in the excised uterus may be washed from a contracting uterus, thereby inducing a state of inactivity. When the first portion of the fluid from these lavages or fluid from Graafian follicles is added to a bath containing a suitable piece of excised inactive uterus, rhythmic contractions ensue. In addition, the original, inactivated uterus may be re-activated by the addition to the bath fluid from the first washings of the perfusion fluid.

At the time they performed their experiments, Brouha and Simmonet were inclined to consider the activating substance to be a hormone associated with oestrus yet one which differed from oestrin. They held this view because oestrin requires some time to effect its well-known growth effects upon the uterus, whereas the activity changes studied by them were sudden in nature. It is impossible to estimate the significance of their results in terms of normal function, inasmuch as no evidence exists concerning the origin or chemical properties of

these activating agents. It is known that very slight changes in either Ca^+ or HCO_3^- concentration have a pronounced effect upon the activity of excised uterine tissue (52). It is evident, therefore, that these findings are to be explained either by assuming the existence of a hypothetical hormone the origin and nature of which remain to be demonstrated or else as being attributable to an artifact of the technic (for the influence of factors of small magnitude see also 197, 96, 100; also, 151).

From what has been said above it is clear that when crude extracts of oestrin are used *in vitro*, conflicting data are obtained, while oestrin given in pure form does not initiate motility in the quiescent uterus *in vitro*.

Convincing proof exists, however, of the replacement effect of pure oestrin in ovary-deficient animals. Significant data in this connection have been obtained in the unanesthetized rabbit by a number of investigators using the uterine fistula (154, 148, 23, 133; dog, 15) and abdominal window (145, 200; monkey, 201) technics in chronic experiments.

Briefly stated, some of the changes which result from oestrin administration (when water is the vehicle for the hormone) in the ovariectomized rabbit may be summarized as follows: the uterus remains quiescent for some hours after injection without initiation of motility (148, 133, 23, 61, 145, 176). Within thirty minutes, the uterus is brightly colored, owing to an active hyperemia (145, 127; see also 26). After several hours, the initial hyperemia subsides slightly although no perceptible increase in myometrial activity is yet evident. After eight to ten hours have passed, motility of the myometrium having a definite rhythm is found. In some animals it may be only commencing at this time while in others it may be quite marked (148, 145; confirmed *in vitro* after preliminary injection of oestrin, 173, 176, 96, 68, 67, 134). During the succeeding hours, up to about the twenty-fourth hour after the first of a series of oestrin injections, the characteristics of the rhythmic motility become more marked, and the contractions more forceful. The time required for a comparable state of motility to develop when the solvent for the hormone is oil is considerably longer than when water is the solvent. Thus, with oil, two to five days are often necessary for significant contractility to develop (unpublished observations of the writer). Upon withdrawal of oestrin the motility subsides over a period of several days with the result that the uterus becomes quiescent in a week or somewhat longer if an oil vehicle has been used. If the administration of oestrin is continued, however, the

uterus remains active for a considerable time (155, 157, 170) but not for an indefinite time (155).

Clearly the rhythmic type of uterine motility which one observes in these experiments is dependent upon the presence of oestrin, and this conclusion is consonant with the broader generalization that the rhythmic motility of oestrus is dependent upon the pure oestrogenic hormone in any of its several forms, whether the hormone is supplied by the animal's own ovaries or by injection (172, 2, 3).

The relation of oestrin to the rhythmic contractions preceding and incidental to labor has been discussed recently elsewhere (96, 167, 156, 23, 171, 176) and, although this subject is important, it does not concern us here since in the present review it is our intention to deal with the underlying mechanisms of periodic uterine motility and its relationship to the mechanism of the sex cycle.

THE NATURE OF THE MYOMETRIAL ACTION OF OESTRIN. In view of the necessity of oestrogenic hormone for coördinated uterine activity typical of oestrus, the question of the means by which oestrin acts upon the myometrium logically arises. Inasmuch as the uterus must be *in vivo* for the pure hormone to exert its characteristic myometrial effect (23, 134, 170, 129, 96, 176, 92, 67), several possible mechanisms suggest themselves to account for the specific action of oestrin. It is possible for instance that oestrin may act directly by bringing about some change in the type or degree of metabolism within the myometrium, or again, it may be that oestrin mediates its effect through some integrating type of action involving the intrinsic innervation and finally, it may be that oestrin mediates its effect by virtue of an action on some endocrine gland or by acting synergistically with some other hormone. It is quite possible also that more than one of these general mechanisms may operate simultaneously. Some of the evidence bearing upon each of these possibilities will now be reviewed briefly.

Posterior hypophysis and oestrin action. The suggestion has been advanced that of all the endocrine glands in association with which oestrin may act particularly at the time of parturition the most likely one is the posterior portion of the hypophysis (172, 141, 168, 169). Thus Blair-Bell and his associates consider (23) that oestrin may stimulate the posterior pituitary lobe in the manner originally suggested by Dixon and Marshall (45). Others consider it possible that oestrin may serve to render the myometrium susceptible to a posterior pituitary hormone (58, 88, 142, 176, 181, 94, 168 and others). The only direct evidence in support of this point of view is the well-known pharma-

codynamic action of posterior pituitary extracts on uterine muscle. While it is now generally agreed that the uterine response one will obtain with posterior pituitary and other oxytocic preparations is dependent in large part upon the prior action of oestrin (147, 199, 74, 153, 96, 73, 134, 170, 144, 145, 28, 136, 23) nevertheless this can hardly be regarded as proof of a physiological synergism between oestrin and some posterior hypophyseal hormone in the normal initiation of rhythmic motility. Rather, it merely shows that oestrin serves to modify the threshold for and character of the responses to posterior pituitary extracts when these are administered as a drug or added to a bath containing excised uterine muscle. Moreover, no deficiency syndrome involving uterine activity has ever been described for the posterior pituitary lobe. Consequently, the replacement effect of extracts of this tissue has never been demonstrated.

On the other hand, there is a considerable body of evidence against the view that the posterior pituitary hormone plays an essential rôle in the initiation of rhythmic motility of the uterus. Briefly stated this evidence is as follows: 1. Oestrin exerts its effect upon rhythmic motility and uterine reactivity in the completely hypophysectomized rabbit in the same low dosage as in the intact animal (158, 174). 2. Pregnant rats, rabbits, and cats with the *pars posterior* and *pars intermedia* of the hypophysis removed experience labor in a normal manner (190, 1, 177). Although vicarious activity of the *tuber cinereum* may be invoked to explain these data, there is no evidence to show that such a mechanism is involved. 3. Oestrin added to a bath containing relatively inactive uteri does not sensitize the myometrium to posterior pituitary extracts subsequently added to the bath (cf. above). This observation is contrary to the earlier work of Bourne and Burn (27) who used impure oestrogenic preparations. It is now established that crystalline oestrin must have acted first *in vivo* for pituitrin to have an appreciable effect upon the uterus isolated subsequently. 4. A hormone of the posterior hypophysis has not yet been identified in the blood or other body fluid or excretion (73, 50, 53, 20, 36, 93; reviews, 74, 172), although claims have occasionally been made that a substance which appears to be somewhat like it may be present at certain times (118, 10, 45, 138, 20). So, although it is maintained by some recent authors that oestrin may be effective especially at parturition by virtue of its action on the posterior hypophysis or in conjunction with a posterior pituitary hormone, the point does not appear to have been proved, and adherence to this view at the present time goes beyond the

established facts. In lieu of evidence that other glands of internal secretion (thyroid, adrenals, etc.) are essential for the myometrial effect of oestrin (23),—other than through an indirect action by way of the anterior hypophysis and thence upon the ovary—one may pass over such possibilities at the present time.

By elimination of this mechanism two general possibilities remain to be considered in the present discussion of the underlying mechanisms by which oestrin specifically initiates and maintains oestrous motility, namely, innervation and alterations in uterine metabolism.

Uterine innervation and oestrin action. Two main types of evidence seem to point to the possible rôle of the intrinsic innervation of the uterus in uterine motility. In the first place, observations on the uterine cervical ganglia of the mouse show that the Nissl substance of these structures is affected by the action of oestrin. In the ovariectomized mouse the Nissl substance is aglomerate and chromophobic but under the influence of oestrogenic preparations this substance becomes conglomerate and chromophilic (24, 101). The significance of these observations has never been ascertained although they suggest that these structural changes may betoken some modification in the functional properties of these cells, whatever those functions may be. Still other evidence suggests that a specialized conduction system, resembling the Purkinje fibers in the heart, may exist in the uterus (91). Such specialized conduction tissue has never been identified in species other than the human, however, and in this form it has only been observed in late gestation. (The innervation of the uterus is reviewed in 43, 62, 76, 23.)

On the functional side the work of Pompen (145) would seem to provide some basis for the view that the intrinsic innervation is intimately concerned in rhythmic uterine contractility, and observations by other technics would seem to confirm this impression (206, 38, 200). Briefly recapitulated, the studies of Pompen show that in the absence of oestrin and in the recently ovariectomized rabbit the uterus is feebly motile, but the activity of one part of the uterus is unrelated to the activity of the other parts of the uterus, either adjacent or remote. Several hours following the injection of oestrin a gradually developing coördination of motility takes place, reaching a maximum when the region of contraction-initiation is at the tubal end of the cornua. The contractions initiated there sweep slowly over the length of the uterus (see also 38, 200), and in this manner the motility of the whole organ is brought under the influence of the tubal portion of the cornua which in

turn is activated by contraction waves originating in the uterine tubes. In the rabbit at this time every fourth or fifth tubal contraction wave continues into the uterus as a major uterine contraction (200).

While there have never been definite grounds for believing that this coördination is a manifestation of nervous regulation, nevertheless this would seem to be a logical assumption. Indeed, on less grounds than this, Blair-Bell is inclined to regard rhythmic contractility as a result, in part at least, of nerve control (23). Moreover, simply because of the complex nature of the coördinated activity of the uterus in labor, Rudolph and Ivy were led to postulate that its basis lay in the controlling influence of the innervation (179) because the contractions found at this time appear to be more complex than certain types of gastro-intestinal activity which are known to have a nervous basis. They adduce no certain evidence in support of this contention, however, and of course the expulsive contractions of the uterus at term are quite different from the contractions one finds in the uterus during oestrus.

Recently, the relationship of the innervation of the uterus to rhythmic activity has been the subject of two studies (163a, 98) which show that despite the above considerations the rhythmic contractions of the uterus at oestrus are independent of the motor sympathetic innervation.

In these studies the fact is brought forth that the uterus of the rabbit possesses no extensive intrinsic motor nerve plexus, while the uterine cervical ganglia (the only regularly occurring cell bodies lying within the myometrium and the ones which undergo modification of their Nissl substance under the action of oestrin) do not connect with motor fibers passing to the uterine cornua (163a). Inasmuch as the classic studies of Langley and Anderson show (119) that there are no inhibitory fibers of sympathetic origin to the uterus of the rabbit (i.e., in the non-pregnant rabbit; the action is altered in pregnancy and pseudo-pregnancy, 180, 51) and that there are no fibers of sacral origin to any of the internal organs of generation in the female rabbit, it is evident that the nature of the uterine innervation of the rabbit is of the most rudimentary sort. While the absence of parasympathetic innervation to the uterus of the rabbit has been doubted (reviews 76, 62) it is conceded generally that the extent of this innervation, if any, is rather limited and its course and components are unknown. As a corollary to these observations on the features of the intrinsic uterine innervation, it has been found that transplanted, denervated uterine cornua (98) and transplanted strips of myometrium (127) respond in a

normal manner both to oestrin administration and the other ovarian hormone, progestin, even in the absence of the *ganglia uterina cervicalia*. These results confirm the many earlier attempts to modify the activity of the uterus by severing its connections with the central nervous system (16, 37, 137; older work, cf. 130), and they extend them by showing the non-essentiality of the intrinsic innervation as well. Numerous attempts to establish the morphological features of this intrinsic uterine innervation by histological means have failed to yield positive results, except to show in the myometrium the occurrence of an abundance of nervous tissue without cell bodies (43, 76, 62, 137, 23, 101).

Parenthetically, it may be noted that rhythmic uterine motility is not peristaltic in nature in the original sense of Bayliss and Starling (19) since intermediation of an intrinsic innervation is not essential to it; rather, oestrous uterine motility consists of simple myometrial contraction waves. It should also be noted in passing that the function or functions which are subserved by impulses arriving at the uterus over its motor nerves are totally unknown at the present time, and the functions of the abundant sensory connections from the uterus remain to be ascertained.

In summary of the discussion of the relationship between intrinsic nervous factors and coördinated rhythmic uterine motility, it may be said that despite several *a priori* considerations favoring the assumption that nervous factors provide the integrating basis for oestrous uterine motility, conclusive evidence shows that this is not so. In the first place, in the rabbit there is no extensive intra-uterine innervation; in the second place, transplanted, denervated uteri respond in a characteristic manner to the motility-stimulating action of oestrin. Evidently, therefore, one must look for changes in the intrinsic metabolism of the uterus to account for this specific myometrium-activating property of oestrin.

Metabolic changes in the myometrium and oestrin action. Since one may not explain the action of oestrin as taking place through either the agency of some endocrine gland or hormone or the innervation of the uterus, that of metabolic changes within the myometrium remains as the third alternative. There is little or no direct evidence to support the view that basic changes of a metabolic nature are responsible for the coördinating action of oestrin on rhythmic uterine contractility. Nevertheless some existing data may point the way to profitable future research in this direction.

One of the earliest observations on periodic uterine activity was that, aside from a variation in the frequency of contractions during the several phases of the sex cycle, one may also demonstrate a difference in the frequency of contractions in the isolated upper (tubal) and the isolated lower (cervical) ends of the uterus respectively (22, 49, 80). This is exemplified when a uterine cornu is divided in the middle and the contractions of the two ends recorded. It is found that the upper part of the uterus contracts with greater frequency than does the lower portion. Wislocki and Guttmacher were aware of this (206) and invoked Child's theory of gradient activity in explanation of their observations. They adapted this theory to uterine musculature directly from its original application to an invertebrate form. To say that this theory applies in the special case of uterine activity is not, of course, an adequate explanation in the absence of proof to support the contention. It should be possible to obtain such proof, however, for methods exist by means of which this hypothesis may be tested. The metabolism of single samples of the uterus has been gauged by oxygen utilization determinations of the myometrium of the mouse (42), rat (103), and sow (105). Likewise, the working power of the uterus has also been examined (104). Each of these studies indicates that at the time when the uterus is under the influence of oestrin, and coinciding with the occurrence of major contractions, both the rate of oxygen utilization and the working power of the uterus are greatest, but no data exist to show the relative oxygen consumption rates of the upper and lower ends of the uterus respectively. It seems quite possible that significant data may be obtained in connection with the point under consideration when such observations as these are made on the two ends of the uterus both in the presence and absence of oestrin and in the presence and absence of motility. Even so, such data (relating as they do to oxygen consumption alone) would merely betoken more deep seated changes in the metabolism of the tissue, especially as regards essential respiratory ferments or as regards the character of the glycogen and inorganic salt metabolism. Johnson, McClosky and Voegtlin, in discussing the importance of oxidation-reduction systems (especially glutathione) in the myometrium to activity, say (97): "Now it should be realized that the determination of the O_2 consumption of living cells furnishes a relatively simple method for the study of the relation of functional to oxidative changes, but information gained by this means has naturally a limited though important significance. After all, estimations of the amount of O_2 consumed and CO_2

produced by living cells furnish only data on the transformation of O_2 by means of cellular constituents into CO_2 and H_2O. No light is thrown on the *mechanism* involved in this complicated process."

HORMONAL INHIBITION OF UTERINE MOTILITY AND REACTIVITY. *Corpus luteum, progestin, and replacement therapy.* It has been suspected for some time that the corpus luteum exerts by hormonic action an inhibitory effect upon uterine contractility (39, 65) but like other phases of reproductive physiology the proof of this assumption has been forthcoming quite recently. From what has been said heretofore it will be recalled that the incidence of markedly reduced uterine motility coincides with the development and life of the corpus luteum. It is natural, therefore, that a causal relationship between the two should be inferred by certain investigators. The possibility that the inactivity of the uterus during early pseudopregnancy in the rabbit may be due to a withdrawal of the stimulating action of oestrin has been considered and found wanting on the grounds that the onset of uterine inactivity is more abrupt than is the waning of rhythmic motility following complete withdrawal of oestrin (148).

Among the first proofs of an inhibited state of the uterus during pseudopregnancy or pregnancy in the rabbit is the observation of Knaus of a low reactivity of the excised uterus to pituitrin and the gradual development of an increasing uterine reactivity to pituitrin, as the corpora lutea retrogress or after they are excised (106, 107, 108, 109, 112, 114). The replacement effect of the corpus luteum hormone progestin was also investigated with the excised uterus of the rabbit after extracts of proven potency became available (40, 4). Workers in many laboratories have participated in the investigation of this point (95, 71, 194, 72, 125, 165, 59, 176, 195; cf. 125 and 7 for references).

In addition to these observations on the reactivity of excised uterine muscle, corroborative evidence on the inhibitory action of corpora lutea and progestin-containing extracts on rhythmical uterine contractility are to be found in the data obtained from unanesthetized rabbits (161, 162, 23, 133, 145, 195, 157, 7, 127) and cows (54). For example, during pseudopregnancy in the rabbit the uterus is quiescent, and is unresponsive to large doses of oestrin administered intravenously, subcutaneously or by both routes combined. As the corpora lutea retrogress or upon excision of the corpora lutea the uterus responds both to the stimulating action of small amounts of oestrin administered within a day of operation (149, 157, 111, 113, 145, 133) as well as to the stimulating action of pituitrin (145, 23, 133). In addition, the motility-

inhibiting action of crude corpus luteum extracts upon naturally-occurring as well as upon oestrin-induced motility has been established (157, 7). As final proof of the inhibitory action of the lutein hormone, progesterone, it is found that the crystalline forms of the hormone are endowed with the same inhibitory property as crude extracts of the corpus luteum possess (7). This same inhibitory action has been found also in the presence of the corpus luteum in the cat, squirrel, cow, sow, and human as well (192, 58, 13, 110, 115, 204, 54).

Species differences in progestin action on the myometrium. Whereas the evidence for the inhibitory action of progestin on the uterus of the rabbit and several other forms is conclusive, a divergent group of data exists for the rat, mouse, and guinea pig (99, 184, 185, 186, 187, 188). When excised uteri from these species are tested for their reactivity to pituitrin they are found to be responsive at all phases of the sex cycle as well as during pseudopregnancy and pregnancy. Thus, contrary to the situation in the rabbit, the myometrium of these species is found not to be inhibited as far as its reactivity to pituitrin is concerned. Nevertheless, the gravid myometrium of the rat will not respond to injection of crystalline oestrin (122, 173). It will be recalled, moreover, that the spontaneous motility of the uterus during the time when there is a corpus luteum in the mouse, rat and guinea pig is found usually to consist of feeble contractions, as opposed to the major contractions which are found at oestrus (cf. 39). Clearly, therefore, there is a modification of activity during this time in these species, although no successful effort has been made to relate this phase of reduced spontaneous motility to the hormonic influence of the corpus luteum. Nor has an attempt been made to ascertain why the reactivity data from these forms are at variance with the extensive observations made on the uterus of the rabbit. When any explanation is offered for the differences just noted, the divergent data are usually attributed to so-called "species differences" (187, 23). This is not an adequate physiological interpretation since the species differences must have a physiological basis of fact even though the nature of the difference is not yet established. Nevertheless some existing data may suggest lines along which fruitful research may be conducted.

In the first place, the endometrium of the rat may be proliferated under the influence of the corpus luteum hormone, but only poorly so (5). In this structural respect the action of progestin simulates in part at least the relatively poor action of progestin extracts found in connection with the reactivity of the myometrium to pituitrin in the rat.

In view of the fact that in the rabbit crystalline progesterone is capable of eliciting both endometrial proliferation and inhibition of spontaneous motility of the myometrium when given in small amounts, while both responses are relatively deficient in the rat, the reason for this deficiency in these two responses in the rat may possibly be attributed to a common causal factor.

It should be mentioned in this connection that in the only *in vitro* studies thus far reported in which crystalline progesterone is used, the pure hormone itself is effective in bringing about a non-reactive state of the uterus of the rabbit when 1 rb.u. (1 mgm.) per day is given for five days (125) but not if less is given. Thus the threshold for the inhibition of pituitrin responses of the uterus *in vitro* is about five times as high as the threshold for endometrial proliferation, while a greater difference exists between the threshold for pituitrin inhibition *in vitro* and *in vivo*, since two-tenths of a rabbit unit of progesterone inhibits rhythmic motility *in vivo* in about five hours. It seems possible, therefore, that with an adequate dosage of progestin in the rat, one may be able to develop a uterus which is non-reactive when tested *in vitro* with pituitrin.

Still a third consideration ought to be mentioned in passing. In the rat and mouse, the oestrous cycles are so short that the corpora lutea of each cycle do not become fully functional in the absence of pregnancy or pseudopregnancy (141). This might account for some of the divergent results described above for this species were it not for the fact that uteri taken even in mid-pregnancy or mid-pseudopregnancy are reactive to pituitrin (184, 185). Since ovariectomy in the rat during pregnancy usually results in abortion[1] (2, 78) and since crystalline oestrin given to rats does not lead to abortion (122, 173) the corpora lutea are probably functional at this time but do not affect the capacity of the uterus, tested *in vitro*, to react to pituitrin. Accordingly, some underlying difference either in the state of the myometrium or in the endocrine balance, or both, is the likely cause of the difference in the *in vitro* uterine responses to pituitrin in the rat and the

[1] Haterius has shown (87) that pregnancy in rats continues after ovariectomy on the fourteenth day of gestation, if adequate placental tissue is left *in situ* and only one fetus remains. Newton has observed that removal of all fetuses in mid-pregnancy, leaving the placentae behind, results in "pseudoparturition" with expulsion of the placentae at the time of expected term (135). Clearly, the placental tissue assists in maintaining the hormonal balance during pregnancy in the rat.

rabbit respectively, when these species are subjected to the action of progestin.

Complementary and supplementary hormonic motility-inhibiting factors. Some very recent observations in the rabbit and ferret dealing with the relationship between the hypophysis and the effectiveness of progestin may also have some bearing on the above point. At least they are of sufficient interest to merit consideration at this time.

It has been found that the amount of progestin which is ordinarily just sufficient to be effective in eliciting full endometrial proliferation (one rabbit unit) is relatively ineffective in this regard when the rabbit is hypophysectomized (159, 175, 143). Larger amounts (circa 4–5 rb.u.) are adequate, however, and elicit full endometrial proliferation when the hypophysis is ablated (175, 160, 189, 11). In addition to this effect of the hypophysis on the threshold for endometrial proliferation, a similar relationship holds for the myometrium-inhibiting action of progestin on pituitrin reactivity (175), and the motility-stimulating action of oestrin (160).

There is still other evidence suggesting a direct inhibitory effect of the pituitary on uterine activity. It has been observed in the intact rabbit that following coitus, but several hours prior to ovulation, the normal oestrous motility virtually ceases and in many instances does in fact cease entirely (161, 145). Similarly, in the absence of coitus but in response to an adequate gonadotropic hormone stimulus, the incidence of ovulation is preceded by onset of uterine quiescence (23, 133, 145, 161, 127). Hence the immediate cause of this effect is independent of any reflex effect associated with coitus. Inasmuch as motility subsides before the Graafian follicles rupture and so before luteinization commences, one must look for some myometrium-inhibiting influence of other than lutein origin as the active agent responsible for this rapid initial quiescence (151). In view of the known release of a pituitary hormone soon after coitus in the rabbit (56, 60, 191) and also in view of the effective inhibiting action of pituitary preparations and of prolan in the ovariectomized rabbit treated with oestrin (to provide motility so that inhibitory effects in the absence of possible corpus luteum function may be demonstrated) (150, 152, 164), it seems plausible to regard the activity of the pituitary body as an important but inadequately appreciated factor in the normal regulation of uterine contractility. The matter should be more extensively investigated, not only in the rabbit, but in other species as well.

It may also be noted that the work of both Newton (135) and of

Haterius (87) shows that placental tissue of the rat effects, or causes to be brought about, an inhibition of oestrus and the attendant uterine contractility. The nature of the motility-inhibiting mechanism associated with the placenta of the rat is totally unknown at the present time, although Haterius suggests without evidence to support it that this tissue secretes progestin. As mentioned above, prolan, which is of human placental origin is capable of inhibiting uterine motility in the rabbit (150) but there is no evidence that prolan is a product of the rat placenta. Judgment concerning the mechanisms involved in these interesting experiments must be withheld for the present.

"Desensin." Despite the fact that the inhibitory nature of the corpus luteum hormone, more especially progesterone, has been clearly established, nevertheless five lines of suggestive evidence exist which indicate the possibility that a second lutein hormone (called "desensin" by Tausk) may also inhibit uterine reactivity to pituitrin. These are as follows: 1, the distribution of two fractions of a corpus luteum extract has given an apparent separation of two active principles; one imparting a high threshold to pituitrin and poor endometrial proliferation and the other resulting in a low threshold of pituitrin reactiveness but giving good endometrial proliferation (178, 71, 72); 2, progestin-containing crystalline material obtained by Fevold and Hisaw effects endometrial proliferation without imparting a high threshold of pituitrin response (59); too low a dosage may have been used, however, (see above and 125); 3, corpora lutea may be produced experimentally which, while bringing about endometrial proliferation, do not inhibit pituitrin responses of the uterus when examined *in vitro* (166); 4, after prolonged lutein activity, endometrial proliferation subsides,[2] but non-reactivity to pituitrin and nerve-excitation remain inhibited (180, 121); and 5, when sufficient oestrin is given concurrently with the corpus luteum hormone, the uterus does not become proliferated (157, 121, 6, 41, 194) but the myometrium is found to be refractory to oestrin *in vivo* (157) and to pituitrin *in vitro* (178). It is equally plausible, of course, to explain the last two points on the basis of differential responses to the single hormone, progestin (157). Nevertheless the foregoing considerations suggest that pituitrin-inhibition *in vitro* and motility inhibition *in vivo* may not be due solely to the corpus luteum hormone, progesterone, but also to a second hypothetical hormone (desensin, Tausk) of luteal origin.

[2] There is one report to the contrary (131).

When, however, the two fractions separated by Tausk, de Fremery and Luchs are tested *in vivo* in the unanesthetized rabbit, both are inhibitory to the motility-inducing property of oestrin, and in addition are inhibitory to the oxytocic factor of pituitrin as well (195). Thus what is the best evidence of two corpus luteum hormones, as judged by *in vitro* tests, is not corroborated when tested in the living, unanesthetized rabbit. It was realized several years ago, in fact, that conclusive proof of two corpus luteum hormones would not be forthcoming until crystalline progestin became available (157), and as already mentioned above, this expectation has been realized (7, 125). Ultimate proof of the multiple effects of the single hormone is obtained by showing that crystalline progesterone is endowed with the capacity to bring about endometrial proliferation, inhibition of rhythmic uterine contractility *in vivo* (7) and uterine reactivity to pituitrin, when suitable dosages are employed (125). It must be added, however, that certain observations remain unexplained, especially those experiments in which a high threshold of response to pituitrin is imparted without an associated proliferation of the endometrium (cf. Tausk et al.).

In *summarizing*, one may say that the preponderance of evidence at the present time indicates that the three uterine reactions considered, viz., endometrial proliferation, inhibition of rhythmic myometrial contractility and desensitization of the uterus to pituitrin, are all attributable to the single hormone, progesterone. In certain species, however, some inherent factor or factors modify these reactions with the result that the endometrial proliferating response is not great and the pituitrin desensitization is lacking even though the corpus luteum is active. Evidence is accumulating to show that the motility-inhibiting action and the pituitrin-reactivity response in the presence of progesterone may be modified under certain circumstances, especially in the absence of the hypophysis, but the relation of the activity of this gland to the normal hormonal regulation of uterine activity needs to be investigated further.

EVIDENCE BEARING ON THE FUNCTION OF THE INTERMITTENT CONTRACTIONS OF OESTRUS. The relationship of uterine motility to the transport of ova and of sperm has been reviewed so recently (84, 140) that it is unnecessary to treat this subject at the present time. Suffice it to say that there is no conclusive proof either for or against the hypothesis that either of these functions is indispensably served by periodic uterine contractions. Another suggested function of cyclic motility is that it is related in some way to the spacing of the ova in the uterus

but aside from the discussion of this subject (39), already referred to, no further evidence bearing upon this aspect of uterine activity seems to have been obtained (see especially 140). It is clear, therefore, in the light of what has been said before, that the functions which are fundamentally served by periodic activity of the uterus during the oestrous cycle have not been defined. More particularly, the most prominent feature of this cyclic motility, the intermittent contractions of oestrus, has not been successfully related to sperm transport, libido, or migration and spacing of the ova.

In dealing with the function which these contractions serve, attention should be called to a correlation which may not be overlooked yet in the past it has been stressed but little. This relationship, to which Loewe has directed attention (review, 124), is that one of the growth phases of the uterus is associated with rhythmic uterine activity and is dependent upon the hormone oestrin;[3] it must be added with equal emphasis that uterine motility is also under the control of oestrin. The two phenomena, uterine growth and activity, take place concurrently in the presence of oestrin and since the two have a common hormonic basis one is compelled to seek the relationship of one to the other. Evidence bearing on this point already exists, and will now be briefly reviewed.

Vascular supply and volume flow of blood through the uterus. Within several days following ovariectomy during oestrus in either rabbits or rats, the rhythmicity and forcefulness of the uterine contractions diminish. This takes place far more rapidly than does atrophy (148, 75). Similarly, when oestrin is administered to a rabbit for about one hundred days uterine motility subsides, uterine vascularity diminishes (i.e., the small vessels become less injected), and atrophy of the uterus follows (155). In view of these and other considerations the tentative conclusion has been drawn that the effect of major uterine contractions is to augment in some way the vascular supply of the non-gravid uterus (155; see also 23). Although investigation of the volume flow of blood through and volume of blood contained within the uterus of the rabbit has been ascertained during gestation (17, 18) similar determinations for the non-gravid uterus have never been made under various hormonic conditions. Several sound physiological considerations indicate that profitable research along these lines may be anticipated.

[3] Uterine growth has also been produced in untreated, ovariectomized rabbits by distention of the uterus with paraffin pellets of suitable size (163b).

It is axiomatic, for instance, in physiology that increased activity (particularly intermittent activity) of a muscle brings about—within limits—an increase in the rate of blood flow through the active tissue (9, 117). In the case under consideration, viz., the uterus, one may also expect to find an increase in the volume flow of blood through the uterus, since Anrep has shown that the volume flow through the vessels of the intestines is much increased by rhythmic contractility and conversely, that blood flow is reduced in the absence of such motility (9).[4]

One might also anticipate, on *a priori* grounds, that the extent of the vascular bed would increase with activity since this is the well-known effect of activity in skeletal muscle. Direct observations on the density of the "vascular population" of the endometrium have been made, however, in which it has been found that conditions in the uterus differ markedly from the situation in skeletal muscle (55). The number of open capillaries and small vessels per square millimeter of average cross section of rabbits' endometrium is about fifty per cent less in motile, active uteri than it is in quiescent uteri of untreated ovariectomized animals. Moreover, the vascular count in quiescent uteri following oestrin administration but prior to the beginning of motility is likewise about one half that of untreated, ovariectomized rabbits. The reason for the decreased vascular count after oestrin administration but before the myometrium becomes active has been ascribed to two coincident phenomena, namely, a marked vasodilatation and an accumulation of interstitial tissue fluid, with subsequent edema. Inasmuch as the extent of this hyperemia (called "blush" by Markee (127) and "peracute effect" by Pompen (145)) is maximal,—that is to say, all the small vessels appear to be open, and inasmuch as the change in permeability precedes the initiation of rhythmic motility, it is clear that major uterine contractions of themselves do not participate in the initial vasodilatation and subsequent reduction in density of the vascular bed per unit of endometrium. Because of Anrep's observations, noted above, however, and with some evidence in support of the conclusion it has been pointed out that intermittent uterine contractions serve to force an increased volume of blood through the uterus per unit of time (55). In addition, it has likewise been pointed out (55) that Drinker and Field have established the fact that intermittent activity of most organs (except the pericardium) augments the flow of lymph from those parts (46), and Andersen has shown that the lymphatic vessels of the

[4] Rhythmic uterine contractions may be so strong at times that they affect the mean level of blood pressure (70).

genital tract are most easily injected at oestrus (8), and so at the time when the intermittent contractions of oestrus occur. The conclusion has been tentatively drawn, therefore, that intermittent uterine contractions serve the double purpose of preventing congestion in the small uterine vessels dilated by the initial, direct action of oestrin, and of removing some of the escaped plasma proteins and tissue fluid by way of the lymphatic vessels; both mechanisms should serve to limit the extent of the edematous changes brought about by the action of oestrin (55).

Relation of blood flow to uterine growth. In connection with the foregoing comments, a question arises concerning the importance of the flow of blood through the uterus for uterine growth. In answer to this question reference must be made to the little quoted experiments of Buchheim and Zaleski (34) and of Bouin and Courrier (25) on the effect of increased vascularity alone on the rate of endometrial and myometrial growth respectively. These investigators observed that when a subchronic vasodilatation is produced (by section of the cervical sympathetic nerve) in an ear of a rabbit containing transplanted endometrial and myometrial tissue, the extent of growth resulting in each transplant is much greater than that which is found in similar transplants to the other ear in which there is normal vasomotor tone. Buchheim and Zaleski state that the degree of growth of the transplant exposed to the augmented blood supply under the conditions of their experiment is almost pathological.

Summary. All in all, therefore, while it should be emphasized that oestrin directly affects the metabolism of the uterus, it may be tentatively concluded that this metabolic action is supplemented by the direct vasodilating effect of oestrin and the consequent effect of this upon the local circulation. This circulation, moreover, is directly aided by the initiation of motility of such a character (i.e., intermittent) that it increases the rate of blood flow through the growing uterus under the stimulating action of oestrin and, at the same time, it serves to limit the effects of a pre-existing hyperemia and edema of the endometrium produced by the direct, primary action of oestrin.

Relation of uterine contractions to menstruation. Before concluding this part of the present discussion, it should be added that the question of the relation of major rhythmic contractions to the menstrual processes may not be adequately appraised at the present time. Much increased uterine motility and reactivity are found just before, incidental, and subsequent to, the onset of menstruation in the human

(110, 204, 132). This coincides with a marked premenstrual rise of oestrin in the blood and urine (69). Nevertheless typical menstruation takes place independently of myometrial activity in endometrial transplants to the eye (128). Moreover, menstruation does not take place in many lower animals in which strong intermittent contractions occur cyclically. However, if major uterine contractions do serve to augment the extent of endometrial blood flow with reduction of congestion and edema, it is inevitable that they must of necessity play a secondary rôle in the menstruation process yet how large a part may not be surmised at the present time.

Before concluding this review, it may be said that we may look forward to the ultimate solution of the problems that have been raised. As these yield, however, to experimental procedures other problems will take their place. One may only speculate for instance on the nature of the mechanism of the inhibitory action of progestin, until the action of its antagonist hormone, oestrin, is revealed. So one might continue for numerous other problems bordering on the subject of this discussion, reference to which for purposes of brevity and clarity have been omitted.

Addendum

For a recent account of cyclic uterine contractility in the human, see a note by R. L. Dickinson (Am. J. Obst. Gynecol. **32**: 828, 1936). Vigorous contractions are noted about the time ovulation is assumed to take place, and moderate contractility is found at the time of menstruation. At other times the uterus is relatively quiescent.

A recent report by Robson (J. Physiol. **88**: 100, 1936) confirms the reports described in the text (7, 145). Robson, too, finds that progesterone, prepared synthetically, renders the myometrium refractory to the stimulating action of oestrone and desensitizes the myometrium to oxytocin. Robson also notes differences in the responses he obtains by *in vitro* and *in vivo* technics, respectively, in rabbits which have been treated with oestrone. This finding is at variance with his earlier work (176) to which reference is made in the text of this review.

Sager and Leonard (Proc. Soc. Exper. Biol. Med. **35**: 242, 1936) have recently reported on the motility-inhibiting action of pregnancy-urine hormone on the myometrium, confirming the report to which reference is made extensively in the text (150).

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